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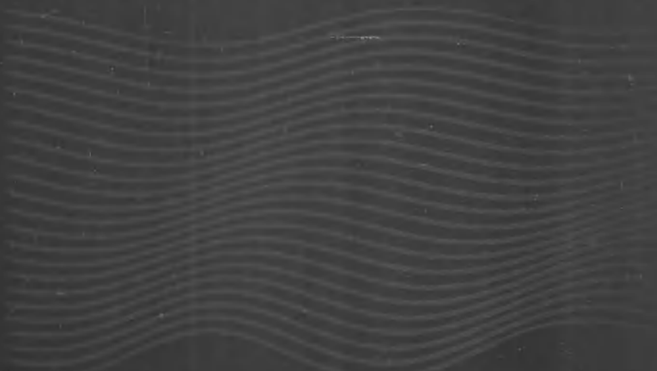
Journal canadien des sciences halieutiques et aquatiques



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A Nonparametric Approach to Generating Inseason Forecasts of Salmon Returns

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Noakes, D. J. 1989. A nonparametric approach to generating inseason forecasts of salmon returns. Can. J. Fish. Aquat. Sci. 46: 2046–2055.

Variations in the size and timing of salmon runs have frustrated attempts to develop models for updating forecasts of returns inseason. The relationship between run size and inseason predictor variables is often nonlinear and changes over time. The nature of these nonlinearities is generally not known and analysts have elected to approximate observed patterns in the data with ad hoc parametric models. Although conceptually attractive, the performance of these models is frequently degraded by their inflexibility and failure to satisfy key model assumptions. In this paper, nonparametric probability density estimation techniques are employed to calculate the total run size conditional on the observed inseason data. Unlike the parametric techniques, the nonparametric approach allows the data to speak for themselves instead of having to merely conform to some arbitrary mathematical model. The approach is easily adapted to handle information from either terminal or guantlet fisheries and can be generalized to compute conditional expectations of various quantities of interest including run size, run timing, or both. Weekly estimates of the number of sockeye salmon (*Oncorhynchus nerka*) returning to the Skeena River, British Columbia, are employed to demonstrate the performance of the model.

Le fait que les montaisons de saumon soient variables en importance et dans le temps nous empêchait de mettre au point des modèles de mise à jour des prévisions des remontes dans la même saison. Souvent, il n'y a pas de rapport direct entre l'importance de la montaison et les variables prédictives applicables à l'intérieur de la saison et ce rapport est modifié avec le temps. On ne sait généralement pas pourquoi ces variations existent et les chercheurs ont choisi d'ajuster les variations observées dans les données à des modèles paramétriques appropriés. Ces modèles sont intéressants sur le plan théorique, mais leur efficacité est souvent amoindrie par leur manque de souplesse et le fait qu'ils ne répondent pas à des hypothèses fondamentales des modèles. Dans cet article, des méthodes de probabilité non paramétriques pour l'évaluation de la densité sont appliquées au calcul de l'importance d'une montaison totale en tenant compte des données relevées dans la saison même. Contrairement aux techniques paramétriques, la méthode non paramétrique laisse les données « parler d'elles-mêmes » au lieu de les modeler à un modèle mathématique arbitraire. Cette méthode se prête bien au traitement des renseignements sur les pêcheries terminales ou d'estuaire et elle peut être appliquée au calcul de prévisions conditionnelles de différentes valeurs d'intérêt, notamment l'importance ou le moment de la montaison ou bien les deux. Des évaluations hebdomadaires du nombre de saumons nerka (*Oncorhynchus nerka*) qui remontent la Skeena, Colombie-Britannique, servent à montrer la performance du modèle.

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Preseason forecasts of salmon returns are employed by fisheries managers to establish tentative fishing plans for each area and species. On the basis of these preseason forecasts, managers decide if enough surplus fish are available to warrant a fishery. These forecasts are of considerable importance to commercial fisherman making decisions about what fishing areas will provide them with the greatest net return and what their investment strategy should be with regard to fishing equipment. The forecasts also provide valuable information to the processing sector of the industry for determining staff, equipment, and logistical requirements for the approaching season.

Equally important, however, are the revised estimates of returns based on inseason information gathered from test or commercial fisheries. Managers rely on preseason and inseason data to schedule openings, to ensure that specified target escapement goals are achieved, and to allocate catch between or among the competing user groups according to predefined arrangements or commitments (Sprout and Kadowaki 1987; Woodey 1987). Errors at any stage of the process may lead to instances where too many or too few salmon are allowed to escape to a subsequent fishery or to spawn. These situations obviously have many short- and long-term economic and biological implications. Given the magnitude and importance of the Pacific salmon fishery to Canada, the United States, and other Pacific rim countries, even modest improvements in our ability to generate preseason and inseason forecasts of salmon returns will result in more efficient and effective utilization of this resource.

Considerable effort has been expended developing models for generating preseason and inseason forecasts of salmon returns or recruitment in general (Beverton and Holt 1957; Cushing 1981; Evans and Rice 1988; Fried and Hilborn 1988; Fried and Yuen 1987; Getz and Swartzmann 1981; Henderson et al. 1987; Jensen 1976, 1985; Mendelssohn 1980; Overholtz et al. 1986; Peterman 1982; Ricker 1954; Rothschild and Mullen 1985; Stocker and Hilborn 1981; Stocker and Noakes 1988). Most of these approaches have concentrated on the development of parametric relationships (linear or nonlinear equations) between observed recruitment and explanatory variables such as spawning stock size or environmental factors. These relationships are often based on a perceived understanding of the physical or biological characteristics of the system being studied. The predictive ability of these parametric models has generally been quite variable with blackbox techniques, such as regression and time series models, often producing superior forecasts of recruitment (Noakes et al. 1989; Stocker and Hilborn 1981; Stocker and Noakes 1988).

A number of probabilistic approaches to recruitment forecasting have also been proposed as alternatives to parametric models (Evans and Rice 1988; Fried and Hilborn 1988; Getz and Swartzmann 1981; Overholtz et al. 1986; Rothschild and Mullen 1985). In these approaches, observed recruitment (and possibly stock size) data are employed to estimate future recruitment without being constrained to a particular functional relationship. The data are, in effect, allowed to speak for themselves instead of having to merely conform to some arbitrary mathematical equation. The likelihood of observing recruitment events (either a value or a range) is estimated using either empirically derived or assumed probability distributions. If the assumptions associated with the parametric models are not satisfied or if the wrong parametric model has been selected, then these probabilistic or nonparametric approaches may produce more accurate forecasts than the competing parametric models.

Because of their robust nature, nonparametric approaches may be particularly useful in situations where the system changes over time. Inseason prediction of salmon runs is perhaps a case in point where variations in run timing and strength result in somewhat chaotic behaviour. For example, one might expect the distribution of run sizes conditional on the catch or return to date to change as the season progresses. During the initial stages of the fishery, there is likely to be considerable ambiguity concerning predictions of run size based on inseason information. Under these conditions, the probability of observing a specific run size might be well approximated by a uniform distribution. As the season progresses, confidence about the run size estimate will increase and an unimodal distribution, such as a Gaussian, may be appropriate. In some instances, it may be possible to use known probability distributions (i.e. uniform, Gaussian, etc.) to estimate the likelihood of various run sizes. However, if there are significant deviations from the assumed probability distribution, then robust empirical approaches to density estimation may provide more realistic assessments of probable run size.

This paper presents a nonparametric approach to forecasting salmon returns inseason. Nonparametric probability density estimation techniques are employed to calculate the total run size conditional on the observed inseason data. The model is easily adapted to handle information from either terminal or gauntlet fisheries and does not require the user to make explicit assumptions concerning run timing. The model can, in fact, be generalized to compute conditional expectations of various quantities of interest including run size, run timing, or both. Data from the Skeena River sockeye salmon (*Oncorhynchus nerka*) fishery are used to demonstrate the model. In addition, a simple procedure is outlined for combining preseason and inseason forecasts of return size in such a way as to gradually increase the weight assigned to the inseason forecast as the season progresses.

Methods

A number of nonparametric regression and data interpretation schemes have been proposed in the literature (see for example Cleveland 1979; Cleveland and Devlin 1988; Cleveland et al. 1988; Friedman and Silverman 1989; Parzen 1962; Rosenblatt 1956, 1971; Silverman 1985, 1986; Spall 1988; Stone 1977; Wahba 1978; Watson 1964; Yakowitz and Szidarovszky 1985). These techniques attempt to capture local characteristics of the data while maintaining a measure of global smoothness. The degree of smoothing is determined by combining some measure of statistical fit with a penalty function related to the roughness of the fitted curve or surface. Small amounts of smoothing will produce estimates (fits) that meander through the data in an attempt to interpolate each observation while increased smoothing will tend towards a more linear representation of the data. Theoretical and practical aspects of these estimators have been discussed in the statistical literature but there have been few empirical studies comparing the relative performance of the various procedures (Friedman and Silverman 1989). Also, as with parametric models, the nonparametric estimator that provides the best fit to the historical data may not produce the best forecasts. Until the predictive ability of the various nonparametric approaches has been compared over a wider range of data types, model selection will necessarily be somewhat judgemental taking into account model performance, implementation, and interpretation.

The model presented in this paper employs a kernel estimator (Parzen 1962; Rosenblatt 1956, 1971) to generate inseason forecasts of run size. The kernel estimator is used to determine the joint probability density function for historical run size and return to date information for each week of the fishery. These estimated density functions are then employed to calculate run sizes conditional on observed inseason data. The kernel estimator is one of the simplest nonparametric methods (Occam's razor) and can be thought of as the continuous analog of multivariate histograms. The kernel estimator, however, provides a better visual representation of the data than the equivalent histogram, highlighting both the central tendencies and spread of the data. This is an important aspect of the model particularly if, as in current practice, the forecasts are to be combined with subjective information (Wright and Ayton 1987).

Kernel nonparametric estimators of density (Parzen 1962; Rosenblatt 1956, 1971) have been extensively investigated and have also found practical application in many fields (see for example Aitchison and Aitken 1976; Aitken and MacDonald 1979; Noakes et al. 1988; Scott et al. 1978; Silverman 1986; Tapia and Thompson 1978; Titterington 1977, 1980; Watson 1964; Wegman 1972; Yakowitz 1985). They can briefly be described as follows. Consider the univariate case where n independent observations, x_i, $i = 1,2,\ldots,n$, have common but unknown density $f(x)$. Then the estimate of $f(x)$, $\hat{f}(x;h)$, based on the kernel $k(\cdot)$ and smoothing parameter h is given by

$$(1)\quad \hat{f}(x;h) = \frac{1}{nh} \sum_{i=1}^{n} k \left(\frac{x - x_i}{h}\right)$$

where $k(\cdot)$ is generally taken to be a probability density function such as the standard normal. The kernel, $k(\cdot)$, is simply a function used to weight the contribution of surrounding data, x_i, to the estimated density at the point of interest, x. The kernel is centred at the point of interest and the weight assigned to each surrounding observation is the magnitude of the kernel at each observation.

In practice, the choice of the kernel, $k(\cdot)$, is not as crucial as is the choice of the smoothing parameter, h, to obtain a reasonable estimate of $f(x)$. Assuming a Gaussian kernel, our estimate becomes

$$\hat{f}(x;h) = \frac{1}{nh}\sum_{i=1}^{n}\frac{1}{\sqrt{2\pi}}\exp[-(x-x_i)^2/2h^2]. \tag{2}$$

The estimated density at any point x is thus a weighted sum of contributions from all observations with the size of the contribution due to point x_i being a function of the distance between x and x_i. The greater the distance, the less point x_i contributes to the estimate.

The smoothing parameter, as the name suggests, determines how smooth our estimate of $f(x)$ will be and selection of an appropriate value for h is an important aspect of the problem. Choosing too large a value for h will result in over-smoothing, while too small a value will lead to an estimate of $f(x)$ that contains more peaks than desired. To illustrate the effect of h, a simple example is presented in Fig. 1. In this exercise, five random points were generated from a normal distribution with mean 0 and variance 1. These points and equation (2) were then employed to estimate the common density, $f(x)$. The procedure involves centring a normal curve (the kernel) at each of the five points and summing the contribution from each curve to obtain an estimate of $f(x)$. The width of each individual normal curve is determined by the smoothing parameter, h, with smaller values of h yielding narrower curves. In Fig. 1a ($h = 0.25$), the individual curves are quite narrow, resulting in an estimate of $f(x)$ that contains four distinct peaks. As h is increased (Fig. 1b–1d), the estimate of $f(x)$ becomes smoother and much less peaked. Since the true $f(x)$ is known to be Gaussian with mean 0 and variance 1, the two extremes, $h = 0.25$ and $h = 2.0$, are instances where the data are under- and over-smoothed, respectively. The most appropriate value of h lies somewhere between these two extremes.

The selection of h has been discussed at some length in the statistical literature (see for example Bowman 1985; Rice 1984; Scott 1986; Scott and Factor 1981; Scott and Terrell 1987; Silverman 1986) and several methods have been proposed. A jackknife-based modified likelihood approach (Duin 1976) is employed in this paper with h selected by maximizing

$$L(h) = \prod_{i=1}^{n} f_i(x_i;h), \qquad h \geqslant 0 \tag{3}$$

where $f_i(x_i;h)$ is the estimate at point x_i based on the samples $\{x_1, x_2, \ldots, x_{i-1}, x_{i+1}, \ldots, x_n\}$. An algorithm developed by

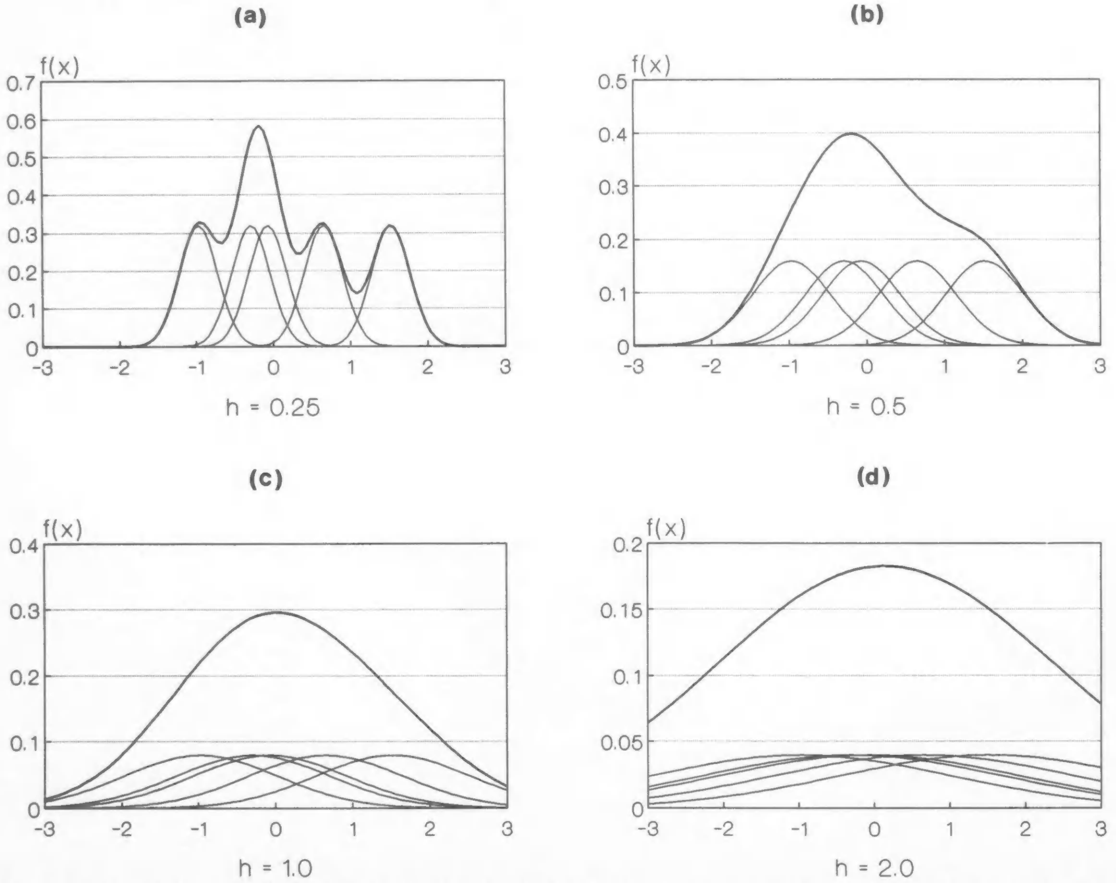

FIG. 1. Example to illustrate the effect of h on the estimation of $f(x)$. Gaussian kernels of varying widths (defined by h) are centred at each of the five random samples (the five smaller individual normal curves). The estimate of $f(x)$ is determined by summing the kernels for each of the five observations to yield the function shown in bold.

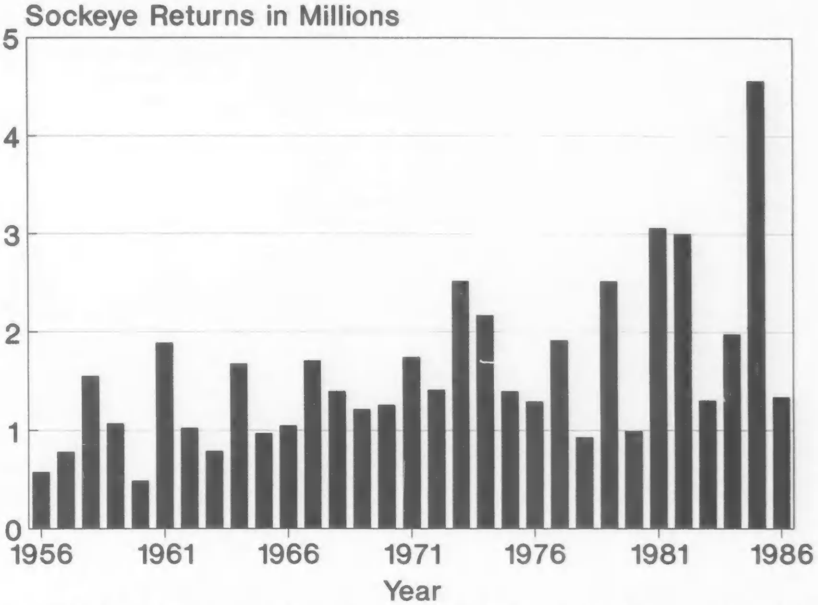

FIG. 2. Sockeye salmon returns (catch and escapement) in millions of fish to the Skeena River, British Columbia, 1956–86. Mean and standard deviation of the return time series are 1 595 000 and 835 000 fish, respectively.

Powell (1964) was employed to obtain estimates of the smoothing parameters used in this paper. Scott and Factor (1981) found the likelihood procedure to work reasonably well although occasional poor estimates resulted from outliers.

The effect of outliers is to inflate the estimate of h resulting in over-smoothing. One possible method of dealing with outliers is to ignore points where $f(x)$ is less than 0.01 max $f(x)$ and rescale the resulting estimate to be a valid probability density function. In the univariate case, this approach is not likely to result in significant errors. However, as d increases, the importance of points in the tails of the distribution grows substantially, and truncating the distribution as described previously may result in inadequate estimates of the density (Silverman 1986, p. 92). In this paper, a less drastic approach has been adopted to deal with outliers. The estimation procedure has been modified to discount, but not completely ignore, the contribution of extreme points to the likelihood function (3). Points within one standard deviation of the mean are assigned a weight of 1, while points beyond this limit are given a weight inversely proportional to the number of standard deviations from the mean to the point.

The multivariate extension of (2) is straightforward yielding an estimate of $f(\mathbf{x})$ as (Cacoullos 1966)

$$(4)\quad \hat{f}(\mathbf{x}) = \frac{1}{n}\sum_{i=1}^{n}\frac{1}{(2\pi)^{d/2}|\Sigma|^{1/2}}\exp[-1/2(\mathbf{x}-\mathbf{x}_i)'\Sigma^{-1}(\mathbf{x}-\mathbf{x}_i)]$$

where $\mathbf{x}$ is a vector of length d and Σ is a common covariance matrix related to the set of multivariate smoothing parameters. To decrease the computational burden involved in evaluating (4), Σ is often replaced by a diagonal matrix such that

$$(5)\quad \hat{f}(\mathbf{x}) = \frac{1}{n}\sum_{i=1}^{n}\frac{1}{h_1\ldots h_d(2\pi)^{d/2}}\prod_{j=1}^{d}\exp[-1/2\{(\mathbf{x}_j - x_{ij})/h_j\}^2]$$

where h_j are the smoothing parameters in each of the d dimensions. This simplication is employed in this paper and appropriate values of h_j are selected using the likelihood approach described above. Although not employed in this study, the process may be further simplified by assuming a single smoothing parameter for all dimensions such that $h_1 = h_2 = \ldots = h_d = h$.

In practice, the variables, $\mathbf{x}$, employed to estimate $f(\mathbf{x})$ may differ by an order of magnitude or more. For example, the cumulative catch or return in the early part of the season may only be a few hundred or thousand fish, while the total run size may be in the millions. The difference in magnitude of these two variables can significantly reduce numerical stability and increase the computational burden associated with estimating the smoothing parameters, h_i, $i = 1, 2$. Typically, one would scale the two variables, in this case cumulative catch or return to date and run size, to overcome these problems. Fukunaga (1972, p. 175) suggested normalizing the data to have a unit covariance matrix, estimating the smoothing parameters for the normalized data, and transforming back to the original domain. This intuitive approach was adopted in this study with the data being normalized by subtracting the estimated means and dividing by the estimated standard deviations prior to estimating the smoothing parameters.

There are, of course, a number of different ways in which this nonparametric model could be used to forecast salmon returns. A preseason forecasting model employing only historical run size information could be developed to provide estimates of total return. In the univariate case ($d = 1$), past data for a single stock would be used to determine the empirical distribution for run size from which the maximum probable return could be estimated. Salmon returns to other river systems or to the same river in previous years could also be incorporated in a multivariate ($d \geqslant 2$) nonparametric model. The expected

TABLE 1. Average, cumulative, and percent catch and return, by week, of sockeye salmon to the Skeena river, British Columbia, 1956–86. Catch and return are in number of fish.

Date	Weekly catch	Cumulative catch	Percent catch	Weekly return	Cumulative return	Percent return
May	1	1	0.00	131	131	0.01
June	17	18	0.00	843	975	0.06
	82	100	0.01	8 039	9 014	0.57
	925	1 025	0.14	23 317	32 330	2.03
	6 521	7 546	1.04	46 858	79 188	4.97
July	20 125	27 671	3.81	78 282	157 470	9.87
	65 683	93 354	12.85	168 781	326 251	20.46
	146 657	240 011	33.03	303 335	629 586	39.48
	205 294	445 305	61.28	362 769	992 355	62.22
	149 583	594 888	81.87	297 567	1 289 922	80.88
August	77 178	672 066	92.49	184 260	1 474 182	92.44
	39 204	711 270	97.89	84 477	1 558 658	97.73
	9 078	720 348	99.13	24 539	1 583 198	99.27
	2 925	723 273	99.54	7 607	1 590 805	99.75
September	2 383	725 656	99.87	3 014	1 593 818	99.94
	543	726 199	99.94	575	1 594 393	99.97
	437	726 636	100.00	436	1 594 829	100.00
	1	726 637	100.00	1	1 594 830	100.00

run size would then be determined conditional on returns to the same river system in years past, or on returns to other river systems in years past, or both.

The addition of inseason information such as cumulative catch in a terminal or single test fishery ($d = 2$) provides a method for generating inseason forecasts of run size conditional on the catch or return observed to date in the current year. Historical run size and catch or return to date information are utilized to estimate $f(\mathbf{x})$, and the current year's run size is then determined by

$$(6)\quad \text{Run size} = \max_{X_1} \hat{f}(\mathbf{x}|X_2 = \text{observed catch to date, current year})$$

where X_1 is run size and X_2 is catch or return to date. Inclusion of information from other sources such as catches in a gauntlet fishery ($d > 2$) is straighforward. The empirical density function, $f(\mathbf{x})$, is simply estimated using (4) or (5) and the expected run size is determined conditional on the catches to date in each of the fisheries being equal to the observed values.

Catch information from gauntlet (test and commercial) fisheries is often used by managers to speculate whether the run is early, late, or on time. Managers examine patterns in historical run time and size information (either in an ad hoc fashion or through simple correlation) to determine the timing and size of the current year's return, to decide on appropriate harvest rates, and to schedule openings. Including catch data from gauntlet fisheries in the nonparametric model provides managers with the same information and a logical framework for comparing these data. The observed catches in each of the fisheries provide an indication of the timing and magnitude of the run, and the simultaneous consideration of these data within the nonparametric model should provide a more realistic assessment of returns. In particular, an advantage of the nonparametric approach is the great flexibility that it provides to model nonlinearities when the nature of the departure from linearity is not obvious as is often the case with fisheries data.

The gauntlet fisheries described in the above inseason forecasting model could, incidently, be separated in space or time or both. The fisheries in the gauntlet could, for example, be the same fishery except at different points in time. In this case, estimated run size for the fishery of interest might be a function of the return or cumulative return in week i as well as the catch or return in the same fishery in previous weeks (i.e. week $i - 1$, $i - 2$, etc.). In this situation, the magnitude and temporal pattern of the weekly returns provide some indication of overall run size and timing. A nonparametric time series model (Noakes et al. 1988; Yakowitz 1985) could be developed to predict run size in this case.

Skeena River Sockeye

Sockeye salmon returns to the Skeena River have averaged 1.6 million fish over the past 30 yr, ranging from a low of 570 000 fish in the mid-1950's to 4.6 million in 1985 (Fig. 2). Returns are intercepted by commercial (seine, gillnet, and troll), sport, and Indian food fisheries and are part of a complex management process involving five Pacific salmon species (*Oncorhynchus* spp.) and steelhead trout (*Oncorhynchus mykiss*). Although considerably smaller than Fraser river sockeye salmon fisheries, the average annual catch of more than 725 000 Skeena river sockeye salmon (Table 1) is of considerable social and economic importance to northern communities in British Columbia. Historically, the Skeena River sockeye salmon fishery strated in the first week of June and lasted until late September. In the past few (5–10) years, however, the commercial fishery has been delayed until July and the fishing season generally ends by early September. Background information about the fishery and its management can be found in Shepherd and Withler (1958) and Sprout and Kadowaki (1987).

Catch and escapement estimates are available on a daily basis from commercial and test fisheries, respectively, from 1956 to 1987. The test fishery is located close (approximately 6 km distance) to the commercial fishery and has in recent years commenced 3 or 4 wk ahead of the first commercial opening. Test fishing is conducted using standardized gear in predefined locations and during tidal events that minimize net movement. Two or three sets are made daily in the test fishery and the results are averaged to provide an estimate of daily escapement. Daily estimates of catch and escapement are then summed to provide estimates of catch, escapement, and total return by week (Table 1).

Weekly cumulative return to date and run size information for the period 1956–86 are employed in this paper to develop a nonparametric inseason forecasting model for Skeena river sockeye. Cumulative return instead of return for a specific week is employed, since variations in run timing can significantly influence returns in any particular week. Employing the return in any specific week as a predictor variable will undoubtedly increase the variability associated with inseason estimates of run size.

Since the total run size will always be at least as large as the run size observed to date, the model is only concerned with predicting the number of additional fish yet to return. Also, the lower $(100 - \alpha)\%$ confidence limit for the estimated run size must be at least equal to the cumulative return to date, and some form of constraint, such as a penalty function, is required to reflect this reality. The simplest method of satisfying both these objectives, and the one employed in this paper, is to subtract the cumulative return to date from the total run size for each week and fit the model to the natural logarithms of these data. The model is employed to forecast the number of salmon yet to return as well as the appropriate upper and lower confidence limits. The return to date is then added to these forecasts to provide estimates of total run size and the confidence interval. Under these conditions, the most probable run size and the upper and lower confidence limits will always be at least as large as the cumulative return to date.

To test the performance of the nonparametric inseason forecasting model, the transformed historical data (1956–86) are used to estimate the joint density function, $f(\mathbf{x})$, for total run size and cumulative return to date for each week of the fishery. Returns by week in 1987 and $f(\mathbf{x})$ are then employed to generate inseason estimates of run size and appropriate confidence intervals conditional on the observed return to date in 1987. Approximate $(100 - \alpha)\%$ confidence intervals are established by finding upper and lower values of run size such that $\alpha/2\%$ of the area under the estimated probability curve is in each tail of the distribution. Preseason and inseason forecasts of returns and their associated confidence intervals are then used to produce a combined forecast of run size. The combined forecast is given by

$$(7) \quad (w \cdot \text{inseason forecast}) + (1 - w) \cdot \text{preseason forecast}$$

where the weight, w, lies in the interval (0,1). The weight assigned to each of the forecasts is calculated using the estimated $(100 - \alpha)\%$ confidence intervals such that

$$(8) \quad w = A \,/\, (A + B)$$

where A is the length of the $(100 - \alpha)\%$ confidence interval for the preseason forecast and B is the length of the $(100 - \alpha)\%$ confidence interval for the inseason forecast. As the season progresses, B should become smaller than A, resulting in more weight being assigned to the inseason forecast.

Results

In 1987, test fishing on the Skeena river began in early June (Table 2). Commercial sockeye salmon fishing commenced one month later and both the commercial and test fisheries continued until the last week of August. Peak returns were on the order of 500,000 to 600,000 fish per week and were observed in late July. The total estimated run size of 2,010,000 fish was slightly above the historical average of 1,595,000 fish but less than half the maximum observed return of 4,558,120 fish recorded in 1985.

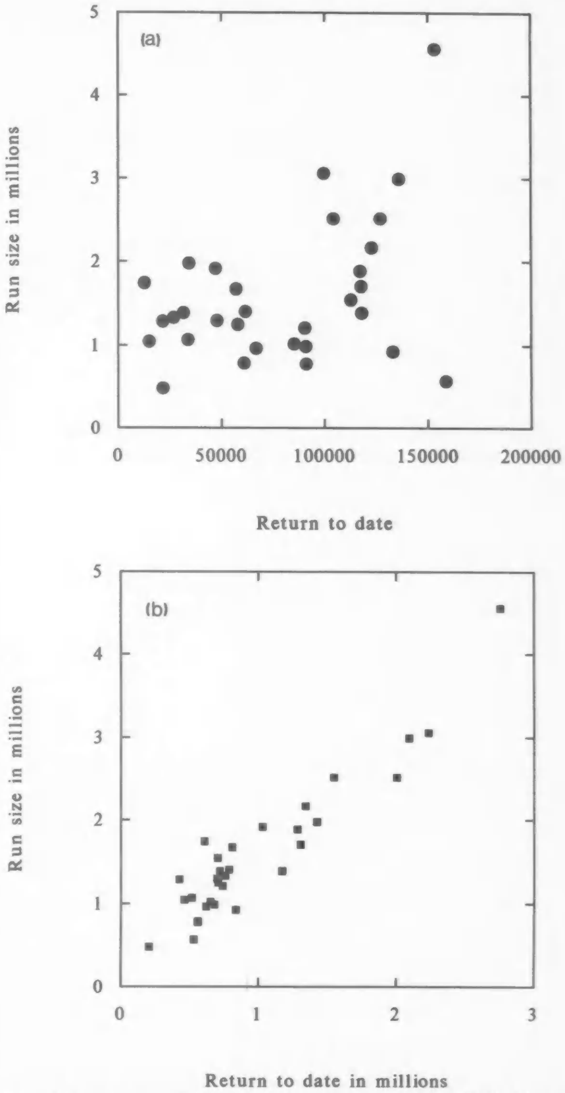

FIG. 3. Run size in millions of fish versus return to date, 1956–86, for (a) the last week in June and (b) the last week in July. Each point represents a single year.

Plots of total run size versus return to date from Skeena river sockeye salmon suggest from a purely visual inspection that one would expect to be able to predict run size with greater precision at the end of July (Fig. 3b) than at the end of June (Fig. 3a). This, of course, should be of little surprise, since one has an extra month's worth of information at the end of July. Since the return to date observations are cumulative, fewer fish

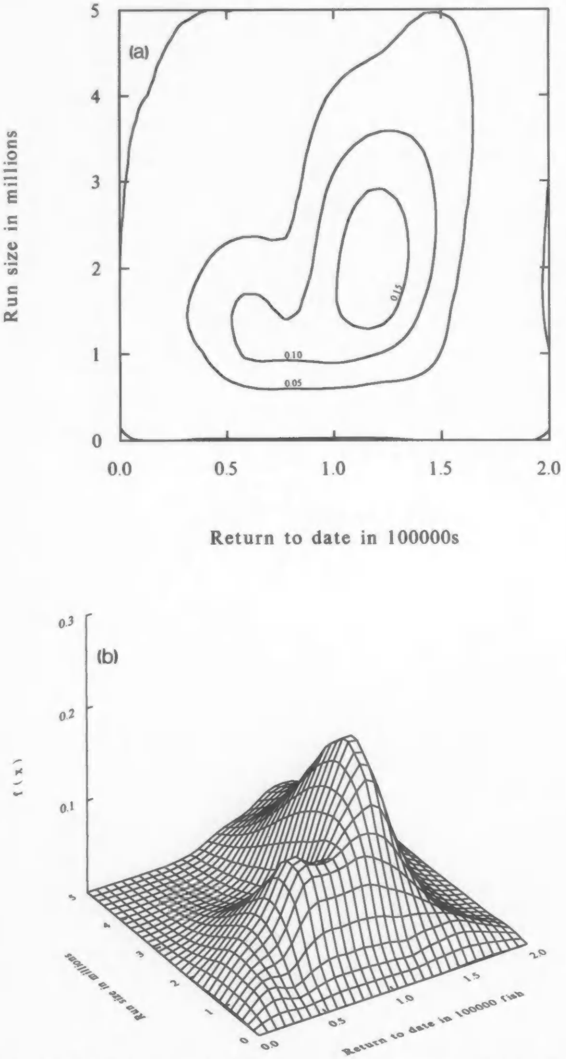

FIG. 4. (a) Contour and (b) three-dimensional perspective plots of the estimated joint density function, $f(\mathbf{x})$, for data from the last week in June, 1956–86.

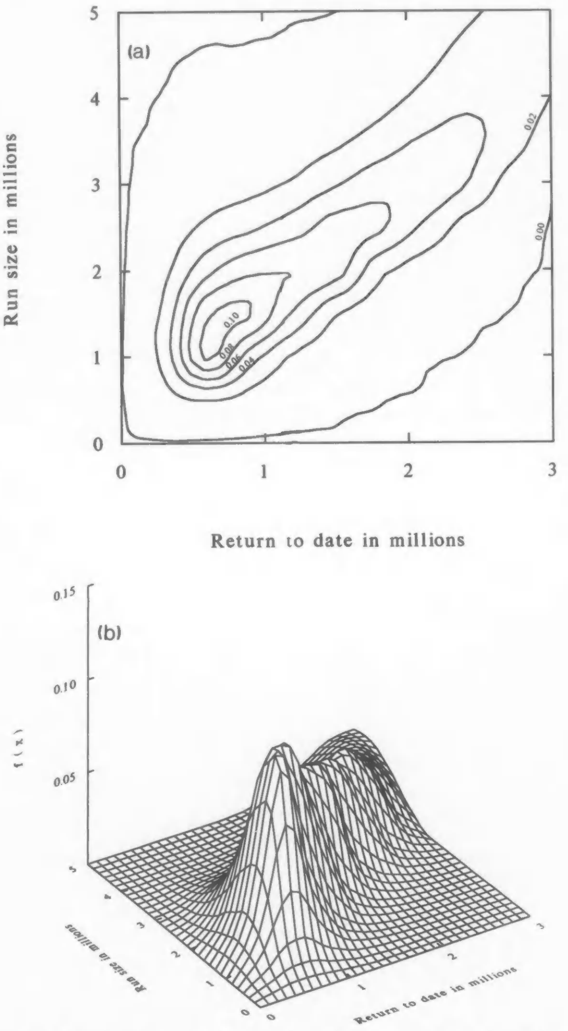

FIG. 5. (a) Contour and (b) three-dimensional perspective plots of the estimated joint density function, $f(\mathbf{x})$, for data from the last week in July, 1956–86.

remain to be accounted for as the season progresses and the data tend toward a straight line with significantly reduced variation.

The June data appear to suggest the existence of two clusters with the separation point for the two groups being approximately 75 000 fish returned to date (Fig. 4). There are no obvious biological reasons to support the occurrence of these two groups and they may simply be an artifact of the analysis. Although the plots are not presented, these two groups are detectable from early June until mid-July but they are less apparent in the plot presented for the last week in July (Fig. 5). However, this does not necessarily imply that the two groups identified in June were artificial. The return data are cumulative and this could, over time, mask the existence of the two groups.

In this study, the historical mean (1 595 000 fish) was employed as the preseason estimate of run size. It may certainly be possible to develop ''better'' models to generate preseason forecasts of salmon returns. However, I elected to use the mean so as not to distract attention from the main focus of the paper which is to consider the development of a nonparametric inseason forecasting model.

The 1987 run of 2.01 million sockeye salmon was 25% larger than the historical mean and preseason forecast of 1 595 000. This low preseason estimate of return resulted in negatively biased combined forecasts until almost the end of the season (Table 2). Similar levels of uncertainty in both the preseason and inseason estimates of abundance resulted in near equal weight being assigned to each of the forecasts during the early part of the season. The weight assigned to the inseason forecast increased gradually until late July at which time it rose sharply.

Table 2. Inseason forecasts of the 1987 sockeye salmon run returning to the Skeena River, British Columbia, approximate 90% confidence intervals (C.I.) for these inseason forecasts, and the relative weight assigned to the inseason forecasts. The inseason forecasts are conditional on observed return to date by week. The combined forecasts are calculated as (1 − weight)·preseason forecast + weight·inseason forecast. The return to date is rounded to the nearest 100 fish, while forecasts and confidence intervals are rounded to the nearest 1000 fish. Preseason forecast is the historical mean return, 1 595 000 fish, with approximate confidence intervals calculated using the estimated standard deviation, 836 000. Actual run size in 1987 was 2 010 000.

Week ending	Cumulative return to date	Inseason forecast	Lower 90% C.I.	Upper 90% C.I.	Weight	Combined forecast
June 6	—	—	—	—	—	1 595 000
13	3 300	1 315 000	502 000	3 253 000	0.51	1 452 000
20	21 600	1 544 000	534 000	3 502 000	0.49	1 570 000
27	72 900	1 020 000	584 000	2 059 000	0.66	1 216 000
July 4	125 000	1 296 000	655 000	2 684 000	0.58	1 422 000
11	298 500	1 419 000	612 000	2 639 000	0.58	1 493 000
18	831 300	1 893 000	1 022 000	3 049 000	0.58	1 768 000
25	1 236 800	1 885 000	1 394 000	2 615 000	0.70	1 798 000
August 1	1 649 300	1 919 000	1 710 000	2 359 000	0.81	1 857 000
8	1 835 200	1 919 000	1 847 000	2 153 000	0.90	1 887 000
15	1 940 900	1 960 000	1 941 000	2 096 000	0.95	1 942 000
22	1 999 700	2 003 000	2 000 000	2 066 000	0.98	1 995 000
29	2 010 000	2 010 000	2 010 000	2 026 000	0.99	2 006 000

This apparent shift in weight corresponded closely to the timing of peak returns of sockeye salmon to the Skeena river (Table 2). With the exception of the third week in June, the inseason forecasts displayed an upward trend converging, as expected, to the actual run size as the season concluded in late August.

The inseason forecasts, as discussed above, are conditional on the observed return to date. The process can be envisaged as examining a cross-section of the joint density function, $f(\mathbf{x})$, with return to date being equal to the current observed value and searching for the maximum probable value in this projection. One such cross-section shows for the June data that the most probable run size (the peak), conditional on the return to date being approximately 73 000 fish (Table 2), is about 1 million sockeye (Fig. 6a). This is certainly consistent with the information displayed in Fig. 3 and 4 because run sizes have historically been in the neighbourhood of a million fish when returns to the end of June were in the 75 000 fish range (Fig. 3a, 4a, and 4b). By the end of July, the most probable run size was estimated at about 2 million fish (Fig. 6b). The greater confidence in the July estimate is reflected in the slight decrease in the spread of the distribution. In both instances (Fig. 6a and 6b), the left tail of the distribution ends abruptly while the right tail decreases more slowly. This asymmetry occurs because the forecasted run sizes were constrained to be at least as large as the observed return to date and because $f(\mathbf{x})$ is actually estimated using the natural logarithms of the data. The forecasts and confidence intervals reported in Table 2 were calculated using the logged data and then transformed back into the original domain.

Discussion

A nonparametric approach to generating inseason forecasts of salmon returns has been outlined. Although the example presented in the paper employed inseason returns to a single terminal fishery, the model can be easily adapted to include catch and/or escapement information from other fisheries the salmon stock may pass through on its way to the spawning grounds (i.e. a gauntlet fishery), catch and escapement from the same fishery in previous time periods, or information about different stocks (from other salmon fisheries) thought to provide some indication of the magnitude and timing of the salmon run of interest. The modelling approach is not limited to forecasting run size and could be used to predict other characteristics such as run timing. An advantage of the nonparametric approach is the great flexibility it provides to model nonlinearities when the nature of the departure from linearity is not obvious as is often the case with fisheries data.

In this paper, I have elected to employ a Gaussian kernel and a modified likelihood approach for estimating the smoothing parameters. These choices are by no means unique and others may find occasions when a less computationally demanding kernel or an alternative to the likelihood approach such as one of the many cross-validation procedures discussed in the statistical literature would better suit their needs. These choices will undoubtedly affect the estimation of $f(\mathbf{x})$ and, in particular, the approximate confidence intervals for the inseason forecasts. Slight changes in the smoothing parameters are unlikely to significantly affect the determination of the extreme points (the maximums) in the distribution but may either lengthen or shorten the tails of the distribution, thus influencing the establishment of approximate confidence intervals.

The results for the example presented in this paper are dependent on the model selected to generate preseason forecasts of sockeye salmon returning to the Skeena river. It is entirely possible that a "better" model than simply using the historical mean could be developed to generate preseason forecasts of returns with perhaps more precision. In this case, greater weight would be placed on the preseason forecast initially and the weight given to the inseason forecast would increase at a slower rate.

Several researchers have found that combining forecasts from two or more competing models will often, but not always, lead to improved accuracy and precision (see for example Bates and Granger 1969; Bordley 1982; Dickinson 1975; Fried and Hil-

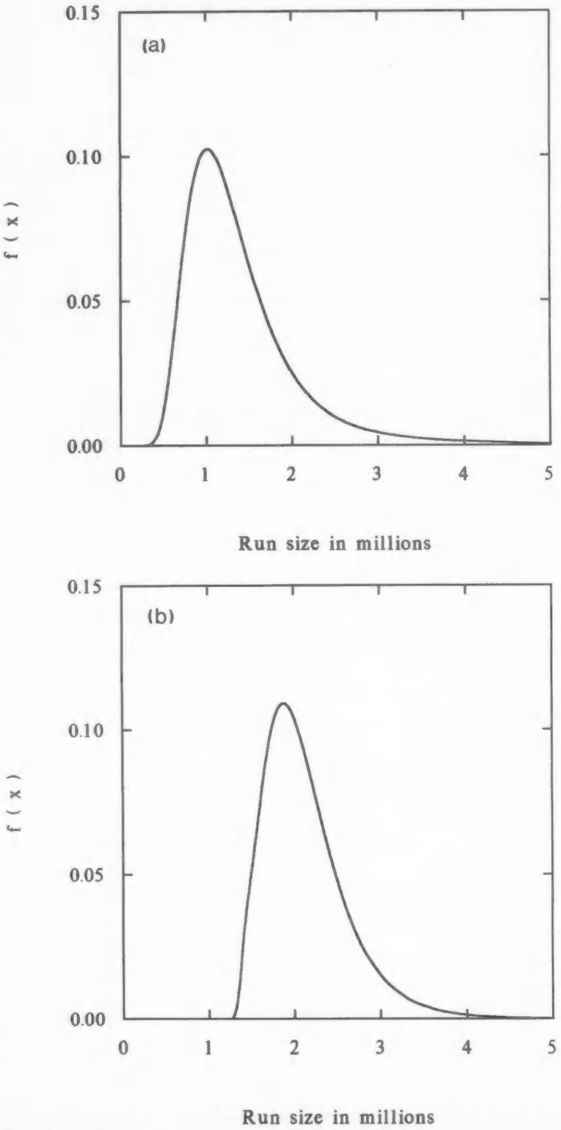

FIG. 6. Estimated probability of total run size conditional on the return to (a) the last week in June 1987 being 73 000 fish and (b) the last week in July being 1 237 000 fish. These plots have been rescaled to integrate to 1.

born 1988; Granger and Ramanathan 1984; McLeod et al. 1987; Newbold and Granger 1974; Winkler and Makridakis 1983). The individual forecasting models may employ different assumptions, data, and expertise and as a result exploit distinct aspects of the problem. The hope is that rather than choosing a single forecasting model the combined forecast will exploit the relative strengths of each method.

Bordley (1982) demonstrated that the best method for combining the individual forecasts will depend on the statistical properties of the forecast errors from each model. If the forecast errors are multivariate normal, the optimal combined forecast is a linear combination of the individual forecasts with the weight assigned to each method calculated using the estimated error covariance matrix (Bates and Granger 1969; Dickinson 1975). If the errors are lognormally distributed, the best combined forecast is a weighted geometric average rather than a simple linear combination of the individual forecasts (Bordley 1982, p. 174). Different distributions will, of course, result in different methods for optimally combining the individual forecasts. If the distributions of the forecast errors are unknown, then one must adopt an empirical approach to determine the most appropriate method to combine the individual forecasts and to estimate the weights to be used in the calculation. Combined forecasts developed under this scenario are likely to be suboptimal.

In this paper, the relative weight assigned to the preseason and inseason forecasts is determined by the length of their respective $(100 - \alpha)\%$ confidence intervals. Other procedures could have been employed but the combined forecast is likely to be suboptimal unless the distributions of the forecast errors are known. One alternative approach would be to split the historical data in two, use the first part of the data to calibrate the preseason and inseason forecasting models, and then employ these models to forecast the second portion of the data. The relative performance of each model for any given week, as measured by some criterion such as the mean squared error of prediction, could then be employed to determine the weight to be given to each forecast. The main drawback of this approach is the short time series generally available. Splitting the series in two means fewer data will be available to estimate model parameters resulting in less confidence in the forecasts produced by these models. For the reasons stated previously, it is unclear whether such an exercise will necessarily provide significantly better estimates of the weights to assign to the preseason and inseason forecasts than employing the simple procedure outlined in the paper.

In this paper, total run size has been estimated conditional on the return to date. Other measures of abundance, such as weekly catch per unit effort (CPUE) or cumulative CPUE, could have been employed in place of return to date in the model. In addition, information on the age or stock composition of the catch could be useful indicators for prediction. The availability of such data and the particular circumstances of each individual fishery will play a major role in determining the precise nature of the information used in the model. In any event, the nonparametric approach provides a convenient and flexible framework within which managers can construct models to forecast run size inseason.

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References

AITCHISON, J., AND C. G. G. AITKEN. 1976. Multivariate binary discrimination by the kernel method. Biometrika 63: 413–420.

Aitken, C., and D. G. MacDonald. 1979. An application of discrete kernel methods to forensic odontology. Appl. Stat. 28: 55–61.

Bates, J. M., and C. W. J. Granger. 1969. The combination of forecasts. Oper. Res. Q. 20: 451–468.

Beverton, R. J. H., and S. J. Holt. 1957. On the dynamics of exploited fish populations. Fish. Invest. Lond. Ser. 2, 19: 533 p.

Bordley, R. F. 1982. The combination of forecasts — A Bayesian approach. Oper. Res. Soc. 33: 171–174.

Bowman, A. W. 1985. A comparative study of some kernel-based nonparametric density estimators. J. Stat. Comput. Simul. 21: 313–327.

Cacoullos, T. 1966. Estimation of a multivariate density. Ann. Inst. Stat. Math. 18: 179–189.

Cleveland, W. S. 1979. Robust locally weighted regression and smoothing scatterplots. J. Am. Stat. Assoc. 74: 829–836.

Cleveland, W. S., and S. J. Devlin. 1988. Locally weighted regression: an approach to regression analysis by local fitting. J. Am. Stat. Assoc. 83: 596–610.

Cleveland, W. S., S. J. Devlin, and E. Grosse. 1988. Regression by local fitting: methods, properties, and computational alogrithms. J. Econometrics 37: 87–114.

Cushing, D. H. 1981. Fisheries biology: a study in population dynamics. University of Wisconsin Press, Madison, WI.

Dickinson, J. P. 1975. Some statistical results in the combination of forecasts. Oper. Res. Q. 24: 253–260.

Duin, R. P. W. 1976. On the choice of smoothing parameters for Parzen estimators of probability density functions. IEEE Trans. Comput. C-25: 1175–1179.

Evans, G. T., and J. C. Rice. 1988. Predicting recruitment from stock size without the mediation of a functional relation. J. Cons. Int. Explor. Mer 44: 111–122.

Fried, S. M., and R. Hilborn. 1988. Inseason forecasting of Bristol Bay, Alaska, sockeye salmon (*Oncorhynchus nerka*) abundance using Bayesian probability theory. Can. J. Fish. Aquat. Sci. 45: 850–855.

Fried, S. M., and H. J. Yuen. 1987. Forecasting sockeye salmon (*Oncorhynchus nerka*) returns to Bristol Bay, Alaska: a review and critique of methods, p. 273–279. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 486 p.

Friedman, J. H., and B. W. Silverman. 1989. Flexible parsimonious smoothing and additive modeling. Technometrics 31: 3–39.

Fukunaga, K. 1972. Introduction to statistical pattern recognition. Academic Press, New York, NY. 369 p.

Getz, W. M., and G. L. Swartzmann. 1981. A probability transition model for yield estimation in fisheries with highly variable recruitment. Can. J. Fish. Aquat. Sci. 38: 847–855.

Granger, C. W. J., and R. Ramanathan. 1984. Improved methods of combining forecasts. J. Forecasting 3: 197–204.

Henderson, M. A., D. Peacock, and R. Goruk. 1987. Evaluation of the reliability of several models used to forecast the size of adult sockeye salmon (*Oncorhynchus nerka*) runs, p. 266–272. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 486 p.

Jensen, A. L. 1976. Time series analysis and forecasting of Atlantic menhaden catch. Chesapeake Sci. 17: 305–307.

1985. Time series analysis and forecasting of menhaden catch and cpue. N. Am. J. Fish. Manage. 5: 78–85.

McLeod, A. I., D. J. Noakes, K. W. Hipel, and R. M. Thompstone. 1987. Combining hydrologic forecasts. J. Water Res. Plann. Manage. 113: 29–41.

Mendelssohn, R. 1980. Using Box-Jenkins models to forecast fishery dynamics: identification, estimation and checking. Fish. Bull. 78: 887–896.

Newbold, P., and C. W. J. Granger. 1974. Experience with forecasting univariate time series and the combination of forecasts. J. R. Stat. Soc. A 137: 131–165.

Noakes, D. J., K. W. Hipel, A. I. McLeod, C. Jimenez, and S. Yakowitz. 1988. Forecasting annual geophysical time series. Int. J. Forecasting 4: 103–115.

Noakes, D. J., D. W. Welch, M. Henderson, and E. Mansfield. 1989. A comparison of alternative methods for generating preseason forecasts of sockeye salmon (*Oncorhynchus nerka*) returns to the Adams and Chilko Rivers, British Columbia, Canada. N. Am. J. Fish. Manage. 9. (In press)

Overholtz, W. J., M. P. Sissenwine, and S. H. Clark. 1986. Recruitment variability and its implications for managing and rebuilding the Georges Bank haddock (*Melanogrammus aeglefinus*) stock. Can. J. Fish. Aquat. Sci. 43: 748–753.

Parzen, E. 1962. On the estimation of a probability density function and the mode. Ann. Math. Stat. 33: 1065–1072.

Peterman, R. M. 1982. Model of salmon age structure and its use in preseason forecasting and studies of marine survival. Can. J. Fish. Aquat. Sci. 39: 1444–1452.

Powell, M. J. D. 1964. An efficient method for finding the minimum of a function of several variables without calculating derivatives. Comput. J. 7: 155–162.

Rice, J. 1984. Bandwidth choice for nonparametric regression. Ann. Stat. 12: 1215–1230.

Ricker, W. E. 1954. Stock and recruitment. J. Fish. Res. Board Can. 11: 559–623.

Rosenblatt, M. 1956. Remarks on some nonparametric estimates of density functions. Ann. Math. Stat. 27: 832–837.

1971. Curve estimates. Ann. Math. Stat. 42: 1815–1842.

Rothschild, B. J., and A. J. Mullen. 1985. The information content of stock-and-recruitment data and its non-parametric classification. J. Cons. Int. Explor. Mer 42: 116–124.

Scott, D. W. 1986. Choosing smoothing parameters for density estimators, p. 225–229. *In* D. M. Allen [ed.] Computer science and statistics: the interface. Elsevier/North Holland Scientific Publishers, Ltd., Limerick, Ireland.

Scott, D. W., and L. E. Factor. 1981. Monte Carlo study of three data-based nonparametric probability density estimators. J. Am. Stat. Assoc. 76: 9–15.

Scott, D. W., A. M. Gotto, J. S. Cole, and G. A. Gorry. 1978. Plasma lipids as collateral risk factors in coronary artery disease — a study of 371 males with chest pain. J. Chronic Dis. 31: 337–345.

Scott, D. W., and G. R. Terrell. 1987. Biased and unbiased cross-validation in density estimation. J. Am. Stat. Assoc. 82: 1131–1146.

Shepherd, M. P., and F. C. Withler. 1958. Spawning stock size and resultant production for Skeena sockeye. J. Fish. Res. Board Can. 15: 1007–1025.

Silverman, B. W. 1985. Some aspects of the spline smoothing approach to nonparametric regression curve fitting (with Discussion). J. R. Stat. Soc. B 46: 1–52.

1986. Density estimation for statistics and data analysis. Chapman and Hall, New York, NY. 175 p.

Spall, J. C. 1988. Bayesian analysis of time series and dynamic models. Marcel Dekker, Inc., New York, NY. 536 p.

Sprout, P. E., and R. K. Kadowaki. 1987. Managing the Skeena river sockeye salmon (*Oncorhynchus nerka*) fishery — the process and the problems, p. 385–395. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 486 p.

Stocker, M., and R. Hilborn. 1981. Short-term forecasting in marine fish stocks. Can. J. Fish. Aquat. Sci. 38: 1247–1254.

Stocker, M., and D. J. Noakes. 1988. Evaluating forecasting procedures for predicting Pacific herring (*Clupea harengus pallasi*) recruitment in British Columbia. Can. J. Fish. Aquat. Sci. 45: 928–935.

Stone, C. J. 1977. Consistent nonparametric regression. Ann. Stat. 5: 595–645.

Tapia, R. A., and J. R. Thompson. 1978. Nonparametric probability density estimation. John Hopkins Press, Baltimore, MD. 176 p.

Titterington, D. M. 1977. Analysis of incomplete multivariate binary data by the kernel method. Biometrika 64: 455–460.

1980. A comparative study of kernel-based density estimates for categorical data. Technometrics 22: 259–268.

Wahba, G. 1978. Improper priors, spline smoothing and the problem of guarding against model errors in regression. J. R. Stat. Soc. B 40: 364–372.

Watson, G. S. 1964. Smooth regression analysis. Sankhya Ser. A 26: 359–372.

Wegman, E. J. 1972. Nonparametric probability density estimation: I. A summary of available methods. Technometrics 14: 533–546.

Winkler, R. L., and S. Makridakis. 1983. The combination of forecasts. J. R. Stat. Soc. A 146: 150–157.

Wright, G., and P. Ayton. 1987. Judgemental forecasting. John Wiley & Sons, New York, NY. 293 p.

Woodey, J. C. 1987. In-season management of Fraser River sockeye salmon (*Oncorhynchus nerka*): meeting multiple objectives, p. 367–374. *In* H. D. Smith, L. Margolis, and C. C. Wood [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96: 486 p.

Yakowitz, S. J. 1985. Nonparametric density estimation and prediction for Markov sequences. J. Am. Stat. Assoc. 80: 215–221.

Yakowitz, S. J., and F. Szidarovszky. 1985. A comparison of kriging with nonparametric regression methods. J. Multivar. Anal. 16: 21–53.

Characterization of Patches Along Transects Using High-Resolution 70-kHz Integrated Acoustic Data [1]

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Nero, R. W., and J. J. Magnuson. 1989. Characterization of patches along transects using high-resolution 70-kHz integrated acoustic data. Can. J. Fish. Aquat. Sci. 46: 2056–2064.

A patch recognition algorithm was applied to high-resolution (1 m vertical and 25 m horizontal) daytime sonar data collected from a 20-km-length transect to a depth of 200 m. The transect was oriented perpendicular to the Gulf Stream frontal zone, 105 km east–northeast ENE of Cape Hatteras, North Carolina, on August 8, 1985. An adaptive high-pass filter was used to identify patches of high-intensity echo strengths. For a broad based averaging "window size" of 13 m deep by 1.4 km long and an echo strength threshold of 1.4 × integrated echo units, patches resemble fine-scale features of the original echogram. A discrimination of patches using sonar statistics from within the patches gave good separation of slope water patches from patches belonging to four other water masses. Slope water patches were characteristically small and of low mean scattering. Large but infrequent targets were present. In the Gulf Stream, by contrast, patches contained more uniformly distributed targets with a higher mean scattering. The observed correlation between echo patches, biological structures, and oceanographic features suggests that the measurement of echo statistics and our patch recognition techniques produce biologically meaningful parameters.

Nous avons traité, à l'aide d'un algorithme de reconnaissance de grappes d'échos, des données sonar à forte résolution (1 m sur la verticale et 25 m sur l'horizontale) recueillies le 8 août 1985, pendant la période diurne, dans un transect de 20 km de long et 200 m de profond orienté perpendiculairement à la zone frontale du Gulf Stream, à 105 km à l'est-nord-est du cap Hatteras, en Caroline du Nord. Un filtre passe-haut adaptatif a permis d'identifier des grappes d'échos de grande intensité. En présence d'une « fenêtre » étendue de 13 m de profond et 1,4 km de long servant au calcul des moyennes et d'un seuil d'intensité d'échos de 1,4 unité d'écho intégré, les grappes se comparent aux éléments à résolution fine de l'échogramme original. Les données sonar ont permis de bien distinguer les grappes d'échos provenant des eaux de la pente continentale de celles provenant de quatre autres types de masses d'eau. Les grappes propres aux eaux de la pente continentale étaient petites et soumises à une faible dispersion moyenne. Des cibles de grandes dimensions ont été détectées, mais peu souvent. En revanche, les grappes d'échos provenant des eaux du Gulf Stream contenaient des cibles réparties plus uniformément et étaient soumises à une dispersion moyenne plus élevée. La corrélation observée entre les grappes d'échos, les structures biologiques et les entités océanographiques semble indiquer que la mesure des données d'échos et nos techniques de reconnaissance des grappes produisent des paramètres utiles sur le plan biologique.

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The patchiness of marine animals and its relationship to rate properties and hydrodynamics is an essential element of dynamic biological oceanography (Legendre and Demers 1984). General ideas and hypotheses have been formulated to address questions concerning diversity, energy flow, connectivity, and system adaptability (Conrad 1985; Margalef 1985). The degree of patchiness plays a central role for all such system-level questions.

A variety of techniques have been used to study patchiness in the ocean, ranging from statistical measures of spatial distribution to Fourier techniques to determine the spectral nature of patchiness (Platt and Denman 1975; Fasham 1978; Legendre and Demers 1984; Mackas 1984). However, all these techniques fail to define the dimensions of a patch, its location, or internal characteristics.

Many studies of oceanic patchiness have analyzed single-dimension transect data (Wiebe 1970; Platt 1972; Fasham and Pugh 1976; Mackas and Boyd 1979; Haury and Wiebe 1982). However, in complex and dynamic oceanic fronts, the greatest amount of spatial complexity is found in two-dimensional cross sections perpendicular to the axis of the front. In such an environment, acoustic transects made with a downward looking sonar are an extremely useful investigative tool and provide a unique viewing perspective for studies of biological patchiness (Greenlaw and Pearcy 1985; Nash et al. 1987).

The use of acoustic transects yields two-dimensional high-resolution data which facilitates the use of nonconventional analysis techniques. Although two-dimensional spectral filters (Ripley 1981) could be used in the identification of patches, they are computationally complex.

In this paper, we develop a simple patch recognition algorithm suitable for processing acoustic backscattering data. This algorithm identifies patches quantitatively while retaining the "picture"-like qualities of the echogram. The algorithm employs two criteria. The first is a two-dimensional high-pass filtering window which determines whether small or large scale patches are detected. It is set depending on the scale of interest. The second is an "adaptive threshold" which determines the minimum echo strength detected. We quantitatively examine the effects of varying both criteria.

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[1] Presented as a paper entitled "Quantification of patches along transects using high-resolution 70-kHz integrated echo data" at the ICES/FAO International Symposium on Fisheries Acoustics, Seattle, WA, June 22–26, 1987.

Methods

Data Acquisition and Processing

Data were collected during a cruise aboard the *R/V Gyre*, using methods similar to that of Nash et al. (1987). In this case we made 10 transects across the near surface frontal zone of the Gulf Stream, 115 km east–northeast ENE of Cape Hatteras, North Carolina, over a 6-d period (August 6–11, 1985). As an example data set, we use the data from one daytime transect made on August 8, 1985, during which a Simrad EY-M 70-kHz echo sounder with an 11° beam width was directed downward. The squared echo signal was integrated over 1-m depth intervals in real time using a programmable echo processor (Powell and Stanton 1983). Echoes were processed to 200 m depth at a rate of 1/3 ping/s. To reduce fluctuations of the reflected sound, integrated echo data were averaged over 4 pings. Finally, corrections for time-varying gain and an upward scaling factor were applied to obtain the average echo intensity $\langle E\rangle$ in aribtrary analog to digital (A/D) converter units suitable for integer operations. These values were stored as a matrix of 200 (depth) by 793 (distance) elements and displayed as volume backscattering strength (Fig. 1) (see corrections below). The transect was approximately 20 km in length giving a horizontal interelement dimension of about 25 m. The top 8 m of data were removed to avoid noise generated by the slow decay of the transmit signal and reflections from near-surface air bubbles and the ship's hull.

For our sonar and A/D converter, the volume backscattering per unit volume (s_v) is given by $s_v = \langle E\rangle \cdot 10^{-10.16}$ where s_v has units of m^{-1}. For density N_i of scatterers having backscattering cross section σ_{bsi}, the mean scattering is

$$s_v = \Sigma\, N_i \cdot \sigma_{\mathrm{bsi}}\ (\mathrm{m}^{-1})$$

and the volume backscattering strength is

$$S_v \equiv 10\ \log_{10}(s_v\, R_1)\ (\mathrm{dB})$$

where R_1 is a reference distance of 1 m (sec. 7.3.2, Clay and Medwin 1977). We sometimes conceptualize s_v as "biomass"; however, unless $N_i \cdot \sigma_{\mathrm{bsi}}$ is proportional to $N \cdot m_i$ where m_i is the mass of the animal, the biomass is not simply related to s_v.

Patch Recognition

Patches are defined as all adjoining super-threshold elements of

$$\langle E\rangle > \langle E\rangle_b \cdot k$$

where $\langle E\rangle_b$ is a measure of background $\langle E\rangle$ calculated from many $\langle E\rangle$ depth–distance elements and k is a threshold multiplier which functions as an additional increment to $\langle E\rangle_b$ (Fig. 2). The term $\langle E\rangle_b \cdot k$ is an "adaptive threshold" which acts as a high-pass filter, removing low-frequency information. The exact size of this information is dependent on the size of the "window" over which $\langle E\rangle_b$ is calculated. The increment k is essential to avoid having a large number of patches occupy the entire data array.

The "adaptive threshold" or $\langle E\rangle_b$ was calculated using a two-dimensional smoothing routine:

$$\langle E\rangle_b = \frac{\sum^{h}\sum^{w} \langle E\rangle}{h \cdot w}$$

which averaged within a rectangular window of h depth by w distance elements. At the edge of the $\langle E\rangle$ array, that portion of the window ($h \times w$) extending beyond the $\langle E\rangle$ array was ignored, thereby preserving the size of the original data. The size of this window ($h \times w$) determined the size of patches detected, with large window sizes generating large patches and small values generating subpatches of larger patches.

To simplify patch structure and eliminate single-element patches, we first included single-element-size gaps occurring in and around patches as part of patches and secondly removed all isolated elements.

Patch Statistics

A variety of location, size, and descriptive statistics were determined (Table 1; Fig. 3). All measures of size are given in units of element-sides. Angle to a nearest neighbor is the absolute angle above or below the horizontal axis expressed in radians. External height and width are a patch's longest dimensions regardless of intervening boundaries, while internal height and width are a patch's longest dimensions found without crossing its boundary. The fractal dimension (Mandelbrot 1983) is determined from the perimeter length to area relationship (Table 1) and is a measure of shape complexity with values of 1 representing square outline shapes and 2 denoting the most complex outline shapes (Turner 1987).

Example Statistical Analysis

We determined the effect of background window size on the patch-finding algorithm by examining the effect of a combination of window heights (7, 13, and 25 elements) and widths (27, 55, and 111 elements) on patch statistics and subsequent discriminant analysis. Window widths were greater than heights because over the same spatial scale, vertical gradients (of biological aggregations) are known to be steeper than horizontal gradients (Legendre and Demers 1984).

To evaluate the effect of threshold (k) adjustment on patch statistics, we examined the behavior of patch statistics as k values were changed at 0.1-unit increments from 1.0 to 1.6 and 0.2-unit increments from 1.6 to 2.0. After selecting a suitable window size and increment k, we ran a discriminant analysis (Fisher 1936) to classify five water masses (see below) using our acoustic measures for each patch as our discriminating variables. Patches were assigned to the Gulf Stream (hot and cold), shelf, slope, and thermocline (between shelf and slope) waters based on concurrent salinity and temperature data (Jech 1986; D. V. Holliday, Tracor Inc., 9150 Chesapeake Dr., San Diego, CA 92123, unpubl. data) from which the generalizations in Fig. 4 were derived. Although only three water masses (shelf, slope, and Gulf Stream) are generally recognized, we made two additional divisions in order to more fully test the ability of our acoustic statistics to differentiate general regions of the front. The effectiveness of the discriminant analysis was evaluated by reclassification of patches using patch statistics not directly

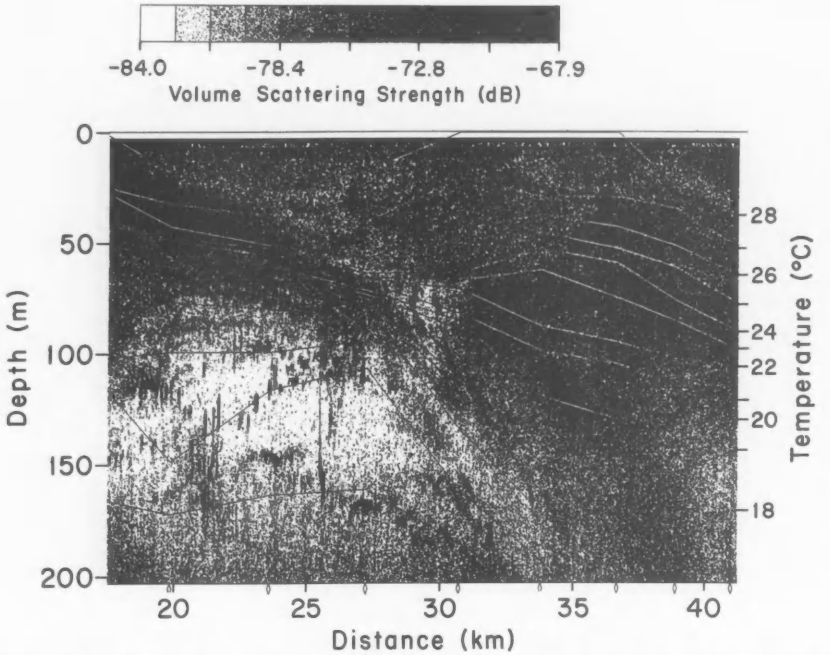

FIG. 1. Echogram depicting volume scattering strength (dB) of transect 8 taken at 09:27 — 12:05 on August 8, 1985. The view is looking northeast with the left-hand side of the figure towards the northwest and the right-hand side towards the southeast. The picture has been reconstructed by computer with fine lines across the transect representing isotherms and open diamonds along the bottom indicating locations of XBT profiles. Offset of 3 m below the surface is for sonar draft.

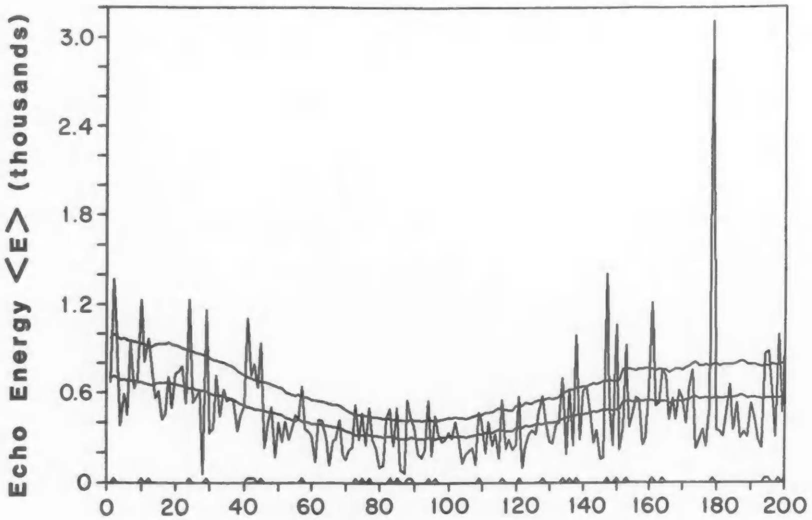

FIG. 2. One-dimensional example of the patch recognition operation with the jagged line representing raw ‹E› data taken from traces 1 to 200 at 15 m depth on transect 8, August 8, 1985. The lower line represents $\langle E\rangle_b$ generated by smoothing ‹E› data with a window width of 11 traces. The upper line denotes a threshold adjustment $\langle E\rangle_b \cdot k$ where $k = 1.4$. Bumps along the horizontal axis denote potential elements of patches ($\langle E\rangle > \langle E\rangle_b \cdot k$). In the actual program, this smoothing operation is carried out over two dimensions.

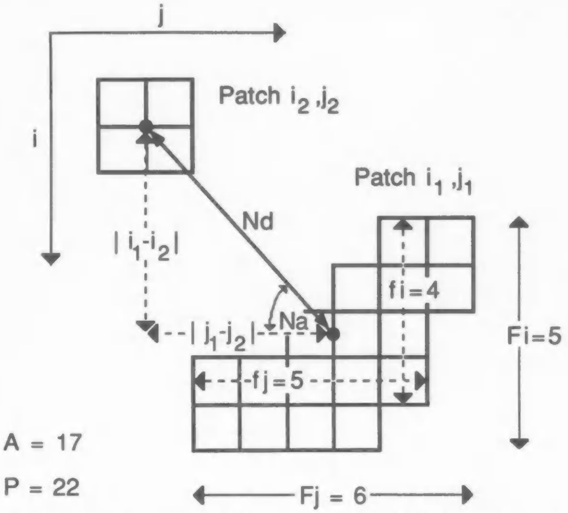

FIG. 3. Example patch and its nearest neighbor (N_n) drawn to demonstrate the calculation of relational and size statistics. Symbols as in Table 1.

TABLE 1. Summary of measurements and statistics determined for each patch within an array of row and column elements. Summations are for the elements within a single patch.

Abbrevation	Variable name	Computation
	Location and oceanographic	
i	Row index	
j	Column index	
T_m	Mean temperature	
T_r	Temperature range	
W	Water mass	
	Relational	
N_n	Nearest neighbor	
N_d	Distance to N_n	$[(i_1 - i_2)^2 + (j_1 - j_2)^2]^{1/2}$
N_a	Angle to N_n	$\tan^{-1} [\lvert i_1 - i_2\rvert / \lvert j_1 - j_2\rvert]$
E_b	Mean $\langle E\rangle_b$	$\frac{\mathrm{k}}{A} \sum \langle E\rangle_b$
	Size and shape	
F_i	External height	See text
F_j	External width	See text
f_i	Internal height	See text
f_j	Internal width	See text
A	Area	Value of 1 per element
P	Perimeter length	Value of 1 per element side
D	Fractal dimension	$\frac{\ln (P/4) \cdot 2}{\ln (A)}$
	Internal features of patches	
E_m	Mean $\langle E\rangle$	$\frac{\sum \langle E\rangle}{A}$
E_{pk}	Peak $\langle E\rangle$	Maximum $\langle E\rangle$ value within a patch
$E_s^{\,2}$	Variance $\langle E\rangle$	$\sum \frac{(\langle E\rangle - E_m)^2}{A-1}$
E_{cv}	Coefficient of variance	$E_S^{\,2}/E_m$
$R_h^{\,2}$	Horizontal roughness	$\sum \frac{(\langle E\rangle_{ij} - \langle E\rangle_{i,j+1})^2}{A-1}$
$R_v^{\,2}$	Vertical roughness	$\sum \frac{(\langle E\rangle_{ij} - \langle E\rangle_{i+1,j})^2}{A-1}$
R_{ch}	Coefficient of horizontal roughness	$R_h^{\,2}/E_m$
R_{cv}	Coefficient of vertical roughness	$R_v^{\,2}/E_m$
E_{sk}	Skewness	Zar 1984, equations 7.7 and 7.8
E_{ku}	Kurtosis	Zar 1984, equations 7.13 and 7.15

associated with a water mass (Table 1). Additional analyses were carried out on a selection of window sizes to investigate the effect of window size on patch classification.

All computations were written in the C language (Kernighan and Ritchie 1978) and run in Unix® using a Masscomp 5500 computer. Multivariate analysis and plotting routines were made using the S statistical programs (Becker and Chambers 1984).

Results

In analyses of patches, a major difficulty is to determine what should be considered a patch. Many differently scaled outcomes of the patch recognition algorithm are possible depending on what recognition criteria are applied to the raw data (Fig. 1). Because large patches occupy large oceanographic features, one may be interested in these large-scale features. Alternatively one may have interest in small patches close to the expected shoal size of the scatterers (Pitcher 1986). These are the smallest meaningful patches that occur within the resolution limits of the data acquisition system.

A visual examination of various threshold window sizes (with $k = 1.4$) indicated that using the smallest window, 7 m deep by 27 elements long (0.7 km), many primary layers in the original acoustic picture were fragmented into many smaller patches (Fig. 5). At the other extreme, a large window of 25 m × 111 elements (2.8 km) yielded patches which incorporated the larger features (Fig. 1). For subsequent analysis we chose the 13 m × 55 element (1.4 km) window size as intermediate and representative of fine-scale (metres to kilometres) features of the original echogram.

Patch statistics responded to changing thresholds and indicate differences between the five water masses (Fig. 6). At thresholds below about 1.3 (Fig. 6), most variables showed inconsistent trends within water masses. At these low thresholds the patch recognition algorithm creates large patches which include a large portion of the transect. These patches may not correctly represent a particular water mass. At higher thresholds ($k > 1.4$), the number of patches, patch area (A), mean $\langle E\rangle$ (E_m), and vertical size become more consistent within water masses (Fig. 6). The low number of patches at $k = 1.0$ is thought to have occurred because a few large patches incorporated many smaller patches. The greatest number of patches occurred with $k = 1.2$. Thresholds above 1.2 resulted in the loss of small patches of

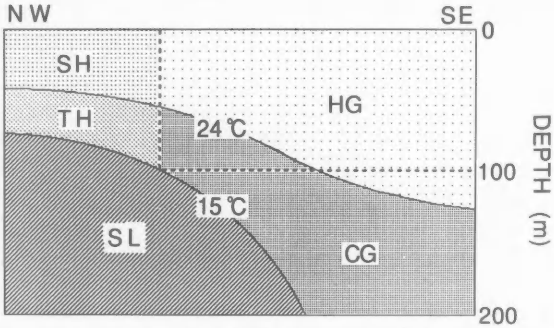

FIG. 4. Diagrammatic representation of the Gulf Stream front with the generalized water masses. SL, slope; TH, thermocline; SH, shelf; HG, hot Gulf Stream; CG, cold Gulf Stream. Where the broken line at 100 m meets the 15°C isotherm, a vertical line is used to separate the shelf and thermocline from the hot and cold Gulf Stream.

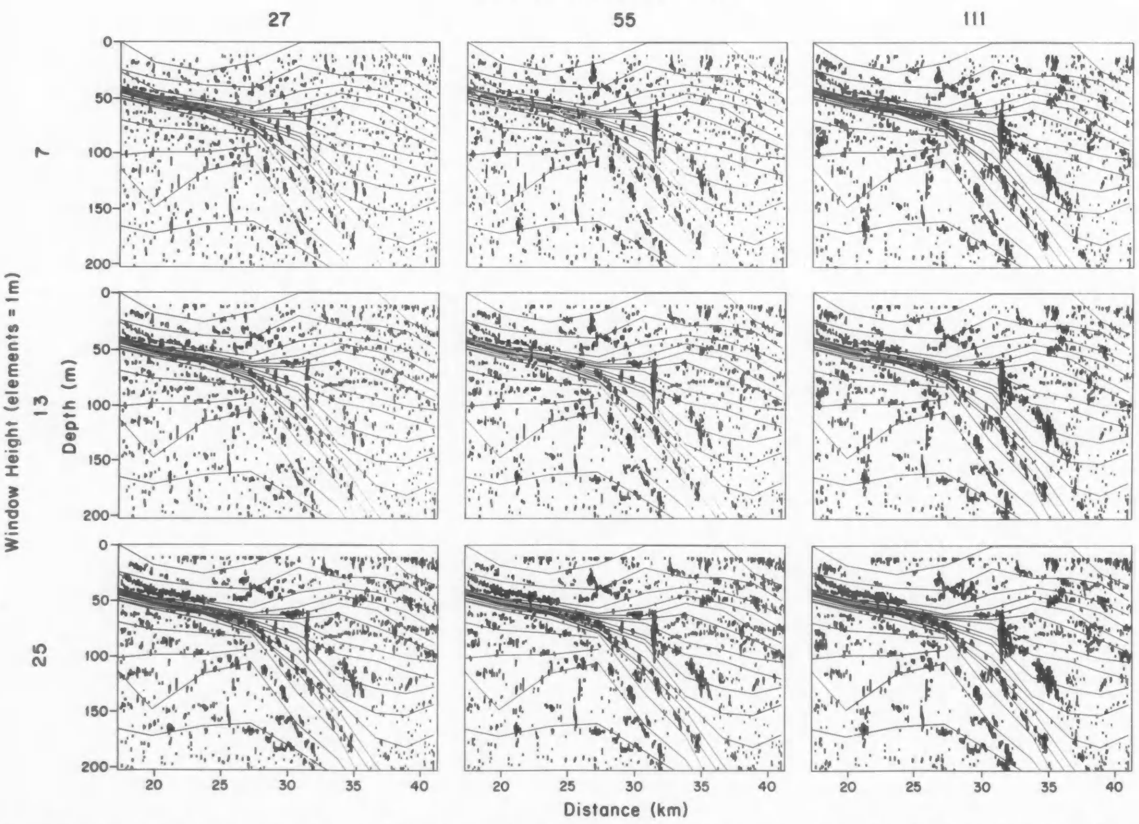

FIG. 5. Black regions indicate patches determined from transect 8 using a variety of smoothing window sizes with a threshold of 1.4 ×. Isotherms are the same as in Fig. 1.

low intensity. Similar trends were evident in patch area, mean ‹E›, vertical size, and the fractal dimension of the largest patch in each water mass. Overall, at high *k*, patches were smaller, of higher intensity, and had a simpler outline shape. Of interest is skewness of the ‹E›. Its average shows marked differences between the Gulf Stream water masses and the slope water side of the front at almost all thresholds (Fig. 6). Higher skewness may indicate a major difference in the biota between the slope water side and Gulf Stream side of the front. If the probability density function (PDF) of the ‹E› is interpreted as the PDF of the echo peak, one can infer a greater abundance of large, solitary scatterers, which comprise nonoverlapping echoes (Stanton 1985), presumably fishes, occurring on the slope side of the front.

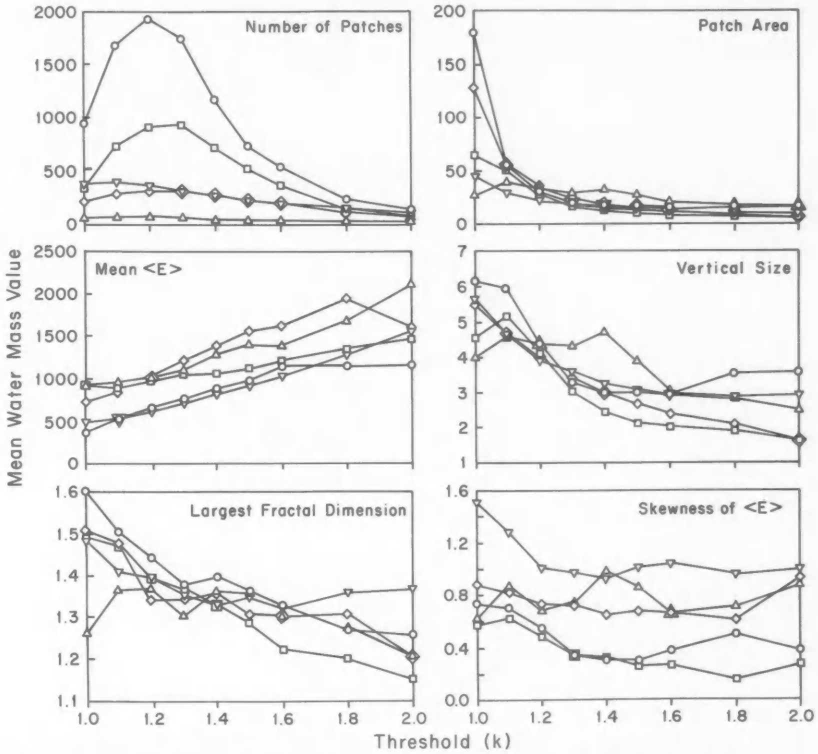

FIG. 6. Effect of changing threshold multiplier (k) on mean values of patch variables and statistics of patches from within each of five water masses. ∇, slope; Δ, thermocline; ◇, shelf; □, hot Gulf Stream; ○, cold Gulf Stream.

Discrimination of water masses by patch statistics was effective, with the best separation obtained between the slope water and the cold Gulf Stream (Fig. 7). The percentage of patches in each water mass correctly reclassified ranged from 31 to 79% (Table 2). One would expect 20%, based on chance alone. Axis 1 is the primary discriminating axis based on a high discriminant correlation of 0.73. In comparison, axes 2 and 3 give discriminant correlations of only 0.31 and 0.26, respectively. Examination of the loadings of patch characteristics on discriminant axis 1 (Table 3) indicates that patches in the slope water (positive end of axis 1) have lower background $\langle E \rangle$ (B_e), higher peak $\langle E \rangle$ (E_{pk}), and smaller areas (A) than those in the other water masses.

Skewness, although previously mentioned as different between water masses, was apparently not weighted highly by the discrimination. This is likely owing to higher variability associated with it than with peak $\langle E \rangle$, background $\langle E \rangle$, or patch area. In addition, at $k = 1.4$, the threshold chosen for the discriminant analysis, skewness for the thermocline is identical to the slope water (Fig. 6) and therefore may have biased against the selection of skewness as a discriminating variable.

A more comprehensive examination of the effect of window size on patch recognition was provided by discriminant analyses of patches within water masses for each of nine window sizes. The relational variable E_b and the size and shape variables A, P, and E_m were consistent in their respective importance, indicating good stability of discriminant axis 1 over all window sizes. Discriminant axis 2 was less stable, but showed good consistency over most window sizes for its primary variable, E_b. Overall, changes in window size appeared to influence patch statistics throughout the echogram equally, such that differences between water masses were consistent.

Discussion

Our definition of patches using arbitrary criteria is not new, but is taken in part from Wiebe (1970) where patches were defined as "a concentration of individuals exceeding the central value in a data set." We have expanded his original concept to two dimensionsand have allowed the "central value" to be adaptive, following overall trends in abundance. We have shown the patch-finding algorithm to be useful for quantifying patchiness that was observed during a single daytime transect within the Gulf Stream.

Comparison of patch variables between large oceanographic features (water masses) has allowed us to determine those acoustic variables most useful in describing fine-scale patchiness of large regions. However, these variables may not be those with the greatest amount of acoustically descriptive information on a fine scale. Based on preliminary principal components analysis of these same data, we suspect that variables associated with internal patch features such as roughness, skewness, kurtosis, and the fractal dimension may have greater utility for describing fine-scale features within a water mass.

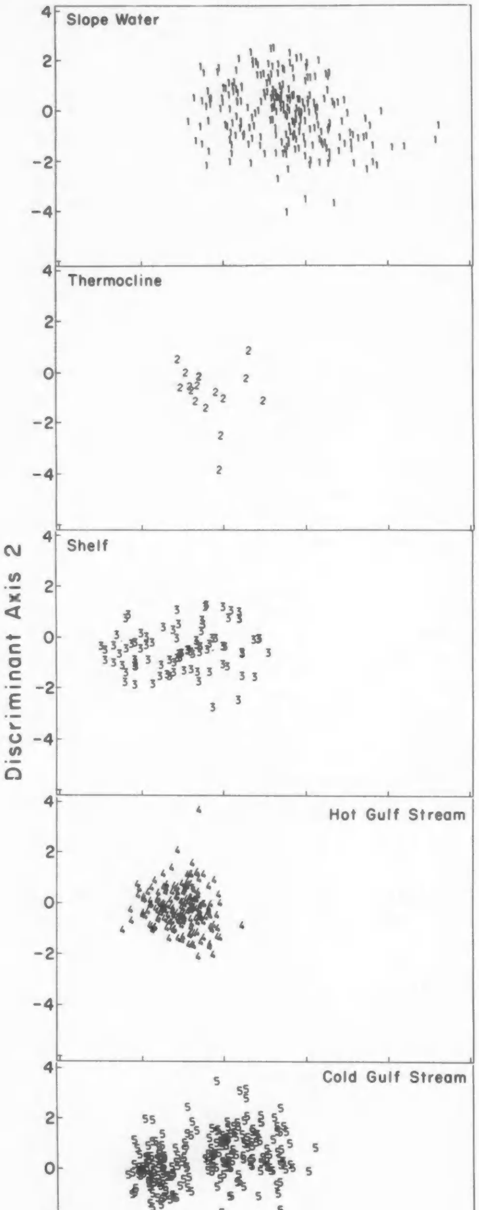

FIG. 7. Scatter plot of the discrimination between five water masses.

Observed dependencies on threshold criteria, in themselves, may have utility as descriptors of the patch characteristics of a water mass; the response to threshold may be more important than choosing the "correct" threshold per se. Overall, both the discriminant analysis and examination of thresholds demonstrate differences between water masses. We believe that these differences reflect changes in the composition and/or distribution of animals across the front.

Much is known of large-scale species differences across the Gulf Stream (Grice and Hart 1962; Backus et al. 1969, 1970, 1977; Jahn and Backus 1976; Haury and Wiebe 1982; Wishner and Allison 1986). Our analyses of acoustic data suggest that the biota within the slope water are different from those of the other four water masses that we assessed. In the slope water, patches are small and intense and occur over a relatively depauperate background, while in the Gulf Stream, patches occur over a background of greater overall biomass. These differences suggest the presence of large fish or dense schools of small fish in the slope water. Night trawl samples (Stanton et al. 1987) have shown small myctophids and small cephalopods to be more abundant at warmer temperatures in the Gulf Stream than at cooler temperatures in the slope water (J. J. Magnuson and R. W. Nero, unpubl. data). In addition, a positively skewed PDF of ‹*E*› suggests a greater occurrence of nonoverlapping echoes and, likewise, large widely dispersed fish in the slope water. We assume that for integrations over small spatial scales, the statistical properties of ‹*E*› approach the statistical properties of the peak detected echo. Overall, with refinement in our ability to isolate particular groups of patches with similar acoustic characteristics, we should be able to gain additional insight into the fine-scale distribution and behavior of organisms at thermal fronts.

Our techniques have applicability in other systems and lead to a methodology where quantitative comparisons of patchiness among disparate pelagic environments, such as lakes and oceans, are now possible. However, we must ensure that in both environments, sonar data are collected in an identical manner and we assume that differential backscattering characteristics of different biotic assemblages do not bias our measurement of patchiness. Meeting these constraints, the spatial patchiness of organisms can be compared. Such comparisons among systems are possible only with a quantitative processing of acoustic data. Nonsonar sampling systems, such as nets, rarely sample both large and small scales with sufficient resolution to allow comparisons.

We have taken full advantage of the two-dimensional "image" provided by acoustics. Only remote sensing of near surface pigments provides a comparable two-dimensional image suitable for studies of patchiness, scale, and extent.

Further refinement in patch size determination, employing acoustic data, may ultimately provide a basis for testing much of the theory on marine patchiness (Ulanowicz and Platt 1985). Our technique integrates horizontal and vertical sampling, a requirement for the testing of several hypotheses on patchiness and its relationship to system adaptability or disturbance (Conrad 1985; Pickett and White 1985), information contained within patterns (Margalef 1985), and scale (Frost et al. 1988).

Our patch characteristics and acoustic measures, identified here as useful water mass discriminators, may also prove useful for the eventual classification and identification of fish schools or aggregations of nekton and macrozooplankton. Because of the simplicity of our algorithm, it could be applied in real-time processing. Recently, Rose and Leggett (1988) have analyzed the echo envelope rather than the average echo intensity. They have defined variables that successfully discriminate between several species of schooling fish. A combination of both methods in real time, the use of averaging techniques to locate schools, and measures of instantaneous echo structure from

TABLE 2. Discriminant analysis classification by 20 patch variables of 758 patches found within five water masses. Percentages in parentheses. Water masses defined in Fig. 4.

	Classified into water mass					
From	Slope	Therm.	Shelf	Hot GS	Cold GS	Total
Slope	130 (53)	34 (14)	51 (21)	26 (11	5 (2)	246 (32)
Therm.	6 (35)	6 (35)	3 (18)	2 (12)	0 (0)	17 (2)
Shelf	18 (21)	11 (13)	37 (44)	13 (15)	6 (7)	85 (11)
Hot GS	33 (19)	13 (7)	33 (19)	55 (31)	43 (24)	177 (23)
Cold GS	1 (0)	5 (2)	2 (1)	42 (18)	183 (79)	233 (31)
Total	188 (25)	69 (9)	126 (17)	138 (18)	237 (31)	758 (100)

TABLE 3. Variable loadings on discriminant axes 1, 2, and 3 (DA1, DA2, DA3). Variables as in Table 1.

Variable	DA1	DA2	DA3
E_{pk}	+1.2	−2.0	−0.20
P	+0.78	−1.2	+6.5
F_j	+0.44	+2.0	+0.12
R_v^2	+0.18	+0.23	−0.97
F_i	+0.097	−0.68	+0.19
E_s^2	+0.092	+0.76	−1.2
f_i	+0.018	+1.3	+0.018
R_{ch}	+0.004	+1.6	−1.7
N_d	−0.023	+0.31	+0.078
N_a	−0.027	+0.31	−0.23
E_{ku}	−0.035	+0.48	+0.006
R_{cv}	−0.063	−0.41	−0.34
E_m	−0.097	+2.6	+1.7
D	−0.13	+0.15	−1.7
R_h^2	−0.14	−2.8	+0.95
E_{sk}	−0.20	−0.14	+0.41
E_{cv}	−0.23	−0.23	+1.8
f_j	−0.23	−1.4	+0.86
A	−1.1	−0.16	−6.0
E_b	−2.2	+0.14	−0.58

within schools should prove beneficial for stock assessment and studies of patchiness.

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References

BACKUS, R. H., J. E. CRADDOCK, R. L. HAEDRICH, AND D. L. SHORES. 1969. Mesopelagic fishes and thermal fronts in the Western Sargasso Sea. Mar. Biol. 3: 87–106.

1970. The distribution of mesopelagic fishes in the equatorial and western North Atlantic. J. Mar. Res. 28: 179–201.

BACKUS, R. H., J. E. CRADDOCK, R. L. HAEDRICH, AND B. H. ROBINSON. 1977. Atlantic mesopelagic zoogeography. Fishes of the western North Atlantic. Mem. Sears. Found. Mar. Res. No. 1, Part 7: 266–287.

BECKER, R. A., AND J. M. CHAMBERS. 1984. S: An interactive environment for data analysis and graphics. Wadsworth Advanced Book Program, Belmont, CA. 550 p.

CLAY, C. S., AND H. MEDWIN. 1977. Acoustical oceanography: principles and applications. John Wiley & Sons, New York, NY. 544 p.

CONRAD, M. 1985. The statistical basis of ecological potentiality, p. 179–186. *In* R. E. Ulanowicz, and T. Platt [ed.] Ecosystem theory for biological oceanography. Can. Bull. Fish. Aquat. Sci. 213: 260 p.

FASHAM, M. J. R. 1978. The statistical and mathematical analysis of plankton patchiness. Oceanogra. Mar. Biol. Annu. Rev. 16: 43–79.

FASHAM, M. J. R., AND P. R. PUGH. 1976. Observations on the horizontal coherence of chlorophyll a and temperature. Deep-Sea Res. 23: 527–538.

FISHER, R. A. 1936. The use of multiple measurements in taxonomic problems. Ann. Eugen. 7: 179–188.

FROST, T. M., D. L. DE ANGELIS, T. F. H. ALLEN, S. M. BARTELL, AND D. J. HALL. 1988. Scale in the design and interpretation of aquatic community research. *In* S. R. Carpenter [ed.] Complex interactions in lake communities. Springer-Verlag, New York, NY. 283 p.

GREENLAW, C. F., AND W. G. PEARCY. 1985. Acoustical patchiness of mesopelagic micronekton. J. Mar. Res. 43: 163–178.

GRICE, G. D., AND A. D. HART. 1962. The abundance, seasonal occurrence and distribution of the epizooplankton between New York and Bermuda. Ecol. Monogr. 32: 287–309.

HAURY, L. R. AND P. H. WIEBE. 1982. Fine-scale multi-species aggregations of oceanic zooplankton. Deep-Sea Res. 29: 915–921.

JAHN, A. E., AND R. H. BACKUS. 1976. On the mesopelagic fish faunas of slope water, Gulf Stream and northern Sargasso Sea. Deep-Sea Res. 23: 223–234.

JECH, J. M. 1986. Physical oceanography of the Gulf Stream from the research cruise Echo Front '85. M.Sc. thesis, University of Wisconsin, Madison, WI. 109 p.

KERNIGHAN, B. W., AND D. M. RITCHIE. 1978. The C programming language. Prentice Hall, Inc., Englewood Cliffs, NJ. 228 p.

LEGENDRE, L., AND S. DEMERS. 1984. Towards dynamic biological oceanography and limnology. Can. J. Fish. Aquat. Sci. 41: 2–19.

MACKAS, D. L. 1984. Spatial autocorrelation of plankton community composition in a continental shelf ecosystem. Limnol. Oceanogr. 29: 451–471.

MACKAS, D. L., AND C. M. BOYD. 1979. Spectral analysis of zooplankton spatial heterogeneity. Science (Wash., DC) 204: 62–64.

MANDELBROT, B. B. 1983. The fractal geometry of nature. W. H. Freeman and Co., New York, NY.

MARGALEF, R. 1985. From hydrodynamic processes to structure (information) and from information to process, p. 200–220. *In* R. E. Ulanowicz, and T. Platt [ed.] Ecosystem theory for biological oceanography. Can. Bull. Fish. Aquat. Sci. 213: 260 p.

NASH, R. D. M., J. J. MAGNUSON, C. S. CLAY, AND T. K. STANTON. 1987. A synoptic view of the Gulf Stream Front with 70-kHz sonar; taking advantage of a closer look. Can. J. Fish. Aquat. Sci. 44: 2022–2024.

PICKETT, S. T. A., AND P. S. WHITE [ED.] 1985. Natural disturbance and patch dynamics. Academic Press, Inc., New York, NY. 472 p.

PITCHER, T. J. [ED.] 1986. The behavior of teleost fishes. Johns Hopkins University Press, Baltimore, MD. 553 p.

PLATT, T. 1972. Local phytoplankton abundance and turbulence. Deep-Sea Res. 19: 183–187.

PLATT, T., AND K. L. DENMAN. 1975. Spectral analysis in ecology. A. Rev. Ecol. Syst. 6: 189–210.

POWELL, L., AND T. K. STANTON. 1983. A programmable microcomputer-based sonar echo processor for real-time processing. IEEE J. Oceanic Eng. OE-8: 280–287.

RIPLEY, B. D. 1981. Spatial statistics. John Wiley & Sons, New York, NY. 252 p.

ROSE, G. A., AND W. C. LEGGETT. 1988. Hydroacoustic signal classification of fish schools by species. Can. J. Fish. Aquat. Sci. 45: 597–604.

STANTON, T. K. 1985. Density estimates of biological sound scattering using sonar echo peak PDFs. J. Acoust. Soc. Am. 78: 1868–1873.

STANTON, T. K., R. D. M. NASH, R. L. EASTWOOD, AND R. W. NERO. 1987. A field examination of acoustical scattering from marine organisms at 70 kHz. IEEE J. Oceanic Eng. OE-12: 339–348.

TURNER, M. G. 1987. Spatial simulation of landscape changes in Georgia: a comparison of 3 transition models. Landscape Ecol. 1: 29–36.

ULANOWICZ, R. E., AND T. PLATT. [ED.] 1985. Ecosystem theory for biological oceanography. Can. Bull. Fish. Aquat. Sci. 213: 260 p.

WIEBE, P. H. 1970. Small-scale spatial distribution in oceanic zooplankton. Limnol. Oceanogr. 15: 205–217.

WISHNER, K. F., AND S. K. ALLISON. 1986. The distribution and abundance of copepods in relation to the physical structure of the Gulf Stream. Deep-Sea Res. 33: 705–731.

ZAR, J. H. 1984. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, NJ. 718 p.

Plasma Cortisol Changes in Seawater-Adapted Mummichogs (*Fundulus heteroclitus*) Exposed to Ammonia[1]

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Spotte, S., and G. Anderson. 1989. Plasma cortisol changes in seawater-adapted mummichogs (*Fundulus heteroclitus*) exposed to ammonia. Can. J. Fish. Aquat. Sci. 46: 2065–2069.

Seawater-adapted mummichogs (*Fundulus heteroclitus*) were exposed to total NH_4-N concentrations of 1, 2, 5, 10, 25, 50, 100, 150, and 200 mg/L for periods of 2, 4, 8, 12, 18, 24, 36, and 48 h. Afterward, the fish were captured quickly and anesthetized with MS-222 before blood was collected. The concentration of total NH_4-N and duration of exposure accounted for, respectively, ~35 and <4% of the observed increase in plasma cortisol. The effective concentration (the concentration of total NH_4-N inducing a mean cortisol response two standard deviations above the mean of the controls) was 47.14 mg/L, corresponding with a mean cortisol level of 13.44 μg/dL. Test concentrations of NH_3-N were calculated from total NH_4-N, pH, salinity, and temperature (constant at 20°C), and the effects of both forms of ammonia on plasma cortisol concentration were compared. Significance was not detected at $p < 0.05$, demonstrating that total NH_4-N and NH_3-N explained the observed changes in cortisol levels similarly. Mean cortisol concentrations of mummichogs anesthetized and sampled in the field were comparable with published values and did not differ significantly from the mean value of captive control fish sampled after the same length of time in MS-222 ($p < 0.05$).

Des fundulus hétéroclites adaptés à l'eau de mer (*Fundulus heteroclitus*) ont été exposés au N-NH_4 total en concentration de 1, 2, 5, 10, 25, 50, 100, 150 et 200 mg/L pendant 2, 4, 8, 12, 18, 24, 36 et 48 h. Par la suite, les poissons ont été rapidement capturés et ils ont été anesthésiés au MS-222 avant les prélèvements sanguins. La concentration de N-NH_4 total ainsi que la durée d'exposition permettent d'expliquer, respectivement, environ 35 % et moins de 4 % de l'augmentation observée du cortisol dans le plasma. La concentration efficace (la concentration de N-NH_4 total qui provoque une réaction moyenne de cortisol supérieure à la moyenne des témoins de deux écarts-types, était de 47,14 mg/L; cela correspond à une concentration moyenne en cortisol de 13,44 μg/dL. Les concentrations d'essai de N-NH_3 ont été dérivées du N-NH_4 total, du pH, de la salinité et de la température (constante à 20°C); les effets des deux formes de l'ammoniac sur la concentration plasmique en cortisol ont été comparés. Il n'y a pas eu de différence significative au niveau $p<0,05$; cela établit que le N-NH_4 total et le N-NH_3 permettent d'expliquer de la même façon les changements observés dans les concentrations en cortisol. La concentration moyenne en cortisol mesurée à partir d'échantillons pris sur place chez des fundulus hétéroclites anesthésiés est comparable aux résultats publiés et elle ne diffère pas significativement de la valeur moyenne des témoins captifs qui ont été échantillonnés après avoir séjourné le même temps dans le MS-222 ($p<0.05$).

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Stress effects in teleost fishes have been classed as primary and secondary (Chavin 1973; Mazeaud et al. 1977; Mazeaud and Mazeaud 1981). Primary effects center on neuroendocrine changes that appear at the systemic level; secondary effects often follow neuroendocrine stimulation. Cortisol, one of the glucocorticosteroid hormones, is considered an indicator of primary stress in bony fishes (Donaldson 1981; Schreck 1981; Passino 1984; Matty 1985).

Aqueous ammonia is one of many stimuli known to induce stress effects in fishes (Holt and Arnold 1983; Shaffi 1980; Smart 1976; Swift 1981; Tomasso et al. 1981). Secondary responses, such as pathologic changes, may not appear for days or weeks (Flis 1968a, 1968b; Schreckenbach et al. 1975; Smart 1976; Thurston et al. 1978, 1986). In contrast, the neuroendocrine system is probably activated immediately (Pickford 1973). This suggests that a change in cortisol concentration may, in some cases, indicate environmental pollution.

Only Tomasso et al. (1981) have described changes in plasma cortisol of a teleost in response to different concentrations of ammonia over time. As a pollutant, ammonia deserves special attention for three reasons. First, most of the amount present in natural waters is biogenic, but only anthropogenic sources are amenable to legislative control. Second, ammonia occurs in two forms, NH_4^+ (ammonium ion) and NH_3 (free ammonia), that appear to differ in degree of toxicity (Erickson 1985). However, only their sum, total ammonia nitrogen (total NH_4-N), can be measured conveniently (Koroleff 1976). Third, data on ammonia toxicity from saline environments are lacking.

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This report describes changes in the plasma cortisol concentrations of seawater-adapted mummichogs (*Fundulus heteroclitus*) during exposure to ammonia over time. We tested the hypothesis that total NH_4-N concentration and duration of exposure correlate positively with plasma cortisol levels. The terms total NH_4-N and ammonia are used interchangeably.

Methods

Mummichogs were captured in minnow traps in the Mystic River estuary of southeastern Connecticut during late summer and early autumn. They were maintained at 20°C under long-day conditions (light exposure > 12 h) in artificial seawater (Spotte et al. 1984; Bidwell and Spotte 1985). Commercial fish food was fed twice daily.

Before the start of the experiment, 119 mummichogs (wild controls) were bled immediately at the capture site to establish resting cortisol levels for comparison with the captive controls. A single fish was removed from the trap, immersed in an anesthetic solution for 1 min, and blood was collected within 3 min of the trap being lifted. Less than 15 s were required to lift and open a trap, remove a fish, and transfer it to the anesthetic. Complete loss of reflex activity (Stage 3 anesthesia, McFarland 1959) took < 30 s. The composition of the anesthetic solution was the same for all wild and captive fish. It consisted of 1 g of 3-aminobenzoic acid ethyl ester methanesulfonate (MS-222) dissolved in 1 L of microfiltered seawater and buffered to pH 7.0 with 4 g of $NaHCO_3$. The solution was warmed to ~25°C immediately before use. Water samples for measurement of ambient (i.e., background) temperature, pH, salinity, and ammonia were collected from the estuary on five occasions when wild fish were captured and bled.

Blood from all fish was obtained by the same procedure. After the caudal fin was severed, whole blood was collected in capillary tubes, expelled into prechilled plastic vials, and placed immediately on ice. Plasma was separated by centrifugation for 5 min at 12 000 × *g* and frozen.

The experiment was divided into trials, each incorporating three concentrations of ammonia (added as reagent-grade NH_4Cl) and an untreated control, all at the same duration of exposure. Trials were conducted at concentrations of 1, 2, 5, 10, 25, 50, 100, 150, and 200 mg total NH_4-N/L over exposure periods of 2, 4, 8, 12, 18, 24, 36, and 48 h. We limited the bioassays to 48 h or less to reduce possible interferences from secondary stress responses. Each combination of concentration and duration of exposure was tested at least twice. The minimum number of fish per trial (i.e., minimum sample size) was estimated from cortisol values of the wild controls ($n = 119$) using equations in Sokal and Rohlf (1981). Four fish were sufficient to detect an average increase in plasma cortisol of 10 μg/dL, a value estimated to represent the magnitude of the cortisol response at the effective concentration of ammonia yet to be determined. Seven fish subsequently were used, although an occasional aquarium contained six or eight. We define effective concentration as the lowest concentration of ammonia that induced a mean cortisol response two standard deviations greater than the mean of the captive controls.

Five days before a trial, seven mummichogs were transferred to each of four bare 38-L aquariums with flowing seawater. Seawater pumped from Long Island Sound to a 1500-L reservoir was heated to 20°C (±2°C) and distributed continuously to the aquariums and then to waste at a rate of 150 mL/min. Illumination was continuous, and food was withheld for 48 h before a trial. The main reservoir also supplied seawater to the captive controls. Three 500-L reservoirs served as sources of ammonia-treated seawater. Before a trial the reservoirs were filled, heated to 20°C (±2°C), and the concentration adjusted with NH_4Cl. Control and treated seawaters were transferred to the aquariums at 150 mL/min.

Immediately before a trial the pH, specific gravity, and ambient concentration of ammonia were measured in the four aquariums and all four reservoirs. At time 0 the treated aquariums were brought abruptly to the test concentrations with NH_4Cl so that each contained a concentration of ammonia equal to its corresponding reservoir. Ambient ammonia concentrations were included in the calculations used to determine adjusted additions. At the end of a trial the fish were captured quickly with a net and immersed together in the anesthetic solution for 1 min before being sampled.

We converted specific gravity to salinity (Zerbe and Taylor 1953). Salinity ranges of the influent seawater and estuarine water were, respectively, 30.1–32.5 and 14.5–31.4‰. The respective pH ranges were 7.70–7.97 and 7.45–8.05. Ammonia was measured spectrophotometrically by an indophenol blue method based on Liddicoat et al. (1975) and modified for standard additions (Spotte 1979). The pH was measured with an electrode pair standardized and calibrated with NBS buffers of pH 7.0 and 10.0.

Plasma samples were thawed on ice and cortisol concentrations determined by radioimmunoassay (No Spin Cortisol, Cambridge Medical Diagnostics, Billerica, MA 01865). The procedure requires 10 μL of plasma. Standards and samples were incubated with I^{125} cortisol tracer in tubes coated with antibody (human serum). After incubation for 45 min at 37°C the contents were aspirated and the radioactivity of bound cortisol in the tubes was read in a gamma counter. Cortisol standards of 0, 1, 2.5, 5, 15, 35, and 75 μg/dL were prepared in triplicate with each set of samples. The detection limit established in our protocol was 1.0 μg/dL. The radioimmunoassay procedure (one series of tubes, $n = 200$) was tested for front-to-back variation; significance was not detected (ANOVA: df $= 3$, p of $F = 0.61983$).

We considered the possibility of masking effects in the treated fish data caused by (1) natural variation in cortisol within the wild population during captivity and (2) stress induced by factors other than the test variables. Potential interfering variables identified in both wild and captive fish were method of anesthesia (see below), standard length, gender, time of day the fish were bled, pH, salinity, and the ambient concentration of ammonia. Absence of significant changes in the plasma cortisol concentrations of the captive control fish was considered evidence that variation observed in the treated fish could be attributed mainly to the test variables.

When the wild controls were sampled, a single fish was bled by one person each time the trap was removed, and all were processed after 1 min in the MS-222 solution. During the trials, seven fish were bled in <12 min by two people. Two methods of anesthesia were used with the captive fish. In both, the fish were left in the MS-222 solution for 1 min, at which time the first two were removed and bled. In Method 1 ($n = 254$) the rest of the fish were left in the anesthetic until they could be sampled, but in Method 2 ($n = 112$) the remaining five were removed and placed between wet paper towels to await sampling.

TABLE 1. Linear regression results for duration of exposure versus cortisol at each test concentration of ammonia. Columns are ammonia concentration, sample size, intercept, slope, *t*-statistic for the independent variable, significance of *t*, and correlation coefficient of the regression.

Total NH_4-N (mg/L)	*n*	*A*	*B*	*t*	*p* of *t*	*r*
1	112	5.642	0.027	0.709	0.4866	0.0675
2	116	6.235	0.085	1.653	0.0971	0.1530
5	118	7.491	0.084	1.489	0.1352	0.1370
10	92	6.286	0.000	−0.007	0.9426	0.0007
25	90	8.190	0.072	1.170	0.2436	0.1237
50	117	14.008	−0.011	−0.174	0.8387	0.0162
100	124	19.239	−0.086	−1.244	0.2132	0.1120
150	119	17.169	0.176	2.163	0.0306	0.1961
200	119	24.068	0.202	2.100	0.0355	0.1906

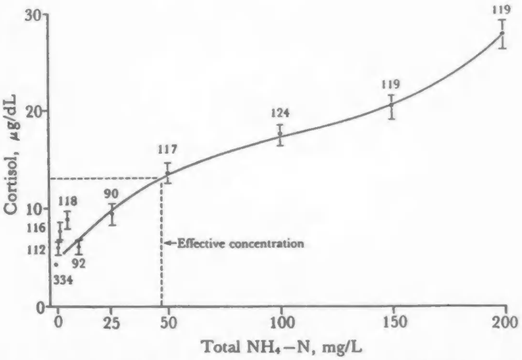

FIG. 1. Cubic regression plot of ammonia versus cortisol for captive controls and treated fish ($n = 1341$). Error bars represent ± SEM; numbers on the curve are pooled values of *n* at each concentration. Effective concentration (47.14 mg/L) is the concentration of ammonia at which the mean cortisol of the treated fish is two standard deviations greater than the mean of the captive controls and correlates with a plasma cortisol concentration of 13.44 μg/dL.

Cortisol levels of the captive control fish ($n = 366$) differed significantly by method of anesthesia (ANOVA: df = 1, *p* of $F = 0.00004$), but the significance was confined to the last two fish sampled using Method 2. These data ($n = 32$) and corresponding data from fish treated with ammonia ($n = 102$) were discarded. The remaining captive control values could be pooled to obtain a common mean (ANOVA: df = 1, *p* of $F = 0.65051$). The mean value of these data ($n = 334$) was 4.42 (±0.25) μg/dL; the mean of the wild controls was 2.08 (±0.27) μg/dL.

Results

When duration of exposure versus plasma cortisol concentration was evaluated by linear regression at each concentration of ammonia, significance was detected only at 150 and 200 mg/L (Table 1, column 6), although even at these levels the effect was weak. A strong, positive correlation between ammonia and cortisol was seen when duration of exposure was disregarded (Fig. 1). The effect of ammonia was significant overall at $p < 0.0001$ (Table 2), but limited to concentrations of 50 mg/L and higher. The effective concentration (Fig. 1) was calculated from the cubic regression model of total NH_4-N versus cortisol of the captive controls and treated fish combined. The value obtained (47.14 mg total NH_4-N/L) correlates with a mean cortisol concentration of 13.44 μg/dL.

Test concentrations of NH_3-N were calculated from ammonia, pH, salinity, and temperature (constant at 20°C) using p*K* values of Khoo et al. (1977). The effects of both forms of ammonia on plasma cortisol concentration were compared using the *F* statistics of corresponding polynomial regressions. In addition, the *t* statistics were determined for the same paired comparisons used to detect significance between correlation coefficients of the regressions (Sokal and Rohlf 1981). Significance was not detected in either analysis at $p < 0.05$, demonstrating that, in this study, total NH_4-N and NH_3-N explained the plasma cortisol response similarly. The range of the coefficients of determination was narrow ($r^2 = 0.352$–0.375) across all polynomial orders, suggesting that exposure to ammonia, expressed either as total NH_4-N or NH_3-N, accounted for ~35% of the plasma cortisol increase. Variation in plasma cortisol increases attributable to duration of exposure was <4% at any ammonia concentration (the largest r^2 value was 0.038).

Mean cortisol levels of the wild and captive control fish removed from the anesthetic solution after 1 min and sampled immediately were not significantly different (two-tailed *t*-test of independent sample means: test $p = 0.446$, $n = 227$). In the case of the captive controls, only values for the first two fish sampled were included in the analysis (Methods 1 and 2, $n = 108$).

Plasma cortisol concentrations of the captive controls were not measurably affected by pH, salinity, and the concentration of ambient ammonia. Cortisol levels of the wild controls were affected by pH and ambient ammonia (ANOVA: df = 1, *p* of $F < 0.05$ for both factors), even though ammonia concentrations in the aquariums typically exceeded those in the estuary ($X = 0.11$ mg total NH_4-N/L, $n = 334$ for the captive controls; $X = 0.07$ mg total NH_4-N/L, $n = 72$ for the wild controls). Standard length, gender, and time of day the fish were sampled showed no correlation with levels of plasma cortisol (ANOVA: df = 1, *p* of $F < 0.05$).

Discussion

Plasma cortisol concentrations did not diminish with extended exposure to any test concentration of ammonia, suggesting no movement toward homeostasis. In fact, duration of exposure had scarcely any effect. Inasmuch as the ammonia concentration was held constant at each calculation, the remaining >96% of the variation in the cortisol concentration was unexplained by the length of time the test fish were exposed to ammonia, regardless of its concentration. Mean plasma cortisol varied, but no pattern was discernible. This resulted partly from

TABLE 2. Post hoc analysis (Scheffé's test of multiple comparisons) of the ANOVA matrix for ammonia versus cortisol ($p < 0.0001$). Lack of significance is confined to multiple comparisons of the lower concentrations, ranges within which the cortisol response is relatively insensitive. The two halves of the table are mirror images. Asterisks represent levels of probability: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Total NH_4-N (mg/L)	1	2	5	10	25	50	100	150	200
1		0.99461	0.85047	1.00000	0.67913	0.00075	0.00000	0.00000	0.00000
2	n.s.		0.99873	0.99684	0.99033	0.02728	0.00001	0.00000	0.00000
5	n.s.	n.s.		0.88454	0.99996	0.21449	0.00009	0.00000	0.00000
10	n.s.	n.s.	n.s.		0.73088	0.00104	0.00000	0.00000	0.00000
25	n.s.	n.s.	n.s.	n.s.		0.38989	0.00028	0.00000	0.00000
50	***	*	n.s.	**	n.s.		0.50824	0.00902	0.00000
100	***	***	***	***	***	n.s.		0.90828	0.00001
150	***	***	***	***	***	**	n.s.		0.00136
200	***	***	***	***	***	***	***	**	

TABLE 3. Cortisol concentrations of control and treated fish. Ammonia is ambient (controls) or predetermined additions. At all concentrations (but not all exposure periods), some specimens appeared to be unaffected, as shown by lower values in the range (column 4). Standard error of the mean increased with ammonia concentration in the treated fish.

	Total NH_4-N (mg/L)	*n*	Range	Mean	± SEM
Wild controls	0.071 (± 0.007 SEM)	72	0.50–10.25	1.801	0.248
Captive control	0.105 (± 0.002 SEM)	334	0.50–39.84	4.420	0.252
Treated fish	1	112	0.50–26.95	6.135	0.578
	2	116	0.50–61.39	7.780	0.799
	5	118	0.50–51.10	9.051	0.891
	10	92	0.50–41.37	6.280	0.703
	25	90	0.50–43.01	9.592	0.940
	50	117	0.50–55.67	3.796	0.906
	100	124	0.50–61.99	7.692	1.061
	150	119	0.50–67.95	20.346	1.262
	200	119	0.50–72.64	27.743	1.450

the variable responses of individual fish (see below). Regression analysis, in which plots of cortisol against time were made at each concentration of ammonia, showed random scatter with slight upward inflections only at the two highest concentrations (Table 1, column 6). The stress response occurred before 2 h, the shortest exposure period tested, and was not diminished at 4 h and beyond.

The most striking observation to emerge is the overall tolerance of seawater-adapted mummichogs to ammonia. As shown in Table 3 (column 4), the cortisol concentration of at least one fish was too low to be detected at every test concentration, although the same pattern was not seen over all exposure periods. This is evidence that the capacity to withstand ammonia toxicity varies with individuals. Even fish with elevated cortisol titers routinely survived levels of ammonia that would have killed many species for which toxicity data are available (U.S. Environmental Protection Agency 1985).

Plasma cortisol values for the wild and captive controls bled immediately after 1 min in the anesthetic solution may be closer to actual resting levels than those published previously for unanesthetized mummichogs. Leach and Taylor (1977) collected serum from mummichogs in a Delaware estuary between March and January and measured cortisol by radioimmunossay. The fish were bled in <3 min without anesthesia, and the mean concentration was 4.24 (± 0.48 SEM) μg/dL. This value compares favorably with the mean of our captive control fish, but is approximately twice that of the wild controls. Leach and Taylor (1977) found no correlation between standard length and cortisol concentration, and we saw none in either the wild or captive control fish.

Ambient ammonia did not affect plasma cortisol levels of the captive controls significantly, even though the aquariums ordinarily contained more ambient ammonia than did water samples from the estuary. Some teleosts can acclimate to ammonia at sublethal concentrations (Lloyd and Orr 1969; Redner and Stickney 1979; Schulze-Wiehenbrauck 1976; Vamos 1963), but most such information has been derived from doseage–mortality studies in which evidence of acclimation is expressed in terms of survival at previously lethal dosages The report of Lang et al. (1987) is an exception. Whether acclimation to ammonia is possible without induction of a primary stress response is unknown.

Leach and Taylor (1977) reported that serum cortisol in mummichogs sampled in the wild did not vary significantly between times of 09:00 and 14:00. Significance was not evident in our wild and captive control data. Wild controls were bled between 09:45 and 15:36 and captive controls between 10:00 and 19:30. However, illumination was constant.

Of all the fish used in the experiment, the wild controls and the captive controls bled after only 1 min in the anesthetic solution probably were affected the least by capture and anesthesia. Data from these groups could be pooled, suggesting that (1) cortisol levels of the captive controls probably were not much different from the wild controls at the time of capture and (2) prior aquarium conditions, always an important consideration in bioassays, were not sufficiently stressful to affect the primary response. Inflections in cortisol concentrations of the treated fish probably were not artifacts, but attributes of the test variables.

The use of physiologic indicators in bioassays has advantages, in part because individual responses can be incorporated into the results (Brown 1973). First, the data represent dosage–response, not dosage–mortality. The former measures the individual response; the latter gives only the cumulative response of all individuals. Second, the effective concentration can be calculated, revealing threshhold levels that define the actual onset of the toxic effect. Third, the potency of the stimulus can be determined from the degree of the response, because each test animal represents a data point. Dosage–mortality tests consider just two classes of data: death and survival. Only the degree of the response associated with a particular level of stimulus can be determined. Brown (1973) emphasized that prediction is an important but seldom obtained objective of bioassays designed to monitor pollution. Prediction of how organisms respond to sublethal levels of toxicants might better be based on reliable primary stress indicators.

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References

BIDWELL, J. P., AND S. SPOTTE. 1985. Artificial seawaters: formulas and methods. Jones and Bartlett, Boston, MA. p. 200–202.

BROWN, V. M. 1973. Concepts and outlook in testing the toxicity of substances to fish, p. 73–95. *In* G. E. Glass [ed.] Bioassay techniques and environmental chemistry. Ann Arbor Science Publishers Inc., Ann Arbor, MI.

CHAVIN, W. 1973. Teleostean endocrine and para-endocrine alterations of utility in environmental studies, p. 199–239. *In* W. Chavin [ed.] Responses of fish to environmental changes. Thomas, Springfield, IL.

DONALDSON, E. M. 1981. The pituitary–interrenal axis as an indicator of stress in fish, p. 11–47. *In* A. D. Pickering [ed.] Stress and fish. Academic Press, London.

ERICKSON, R. J. 1985. An evaluation of mathematical models for the effects of pH and temperature on ammonia toxicity to aquatic organisms. Water Res. 19: 1047–1058.

FLIS, J. 1968a. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. Part I. Effects of toxic concentrations. Acta Hydrobiol. 10: 205–224.

1968b. Anatomicohistopathological changes induced in carp (*Cyprinus carpio* L.) by ammonia water. Part II. Effects of subtoxic concentrations. Acta Hydrobiol. 10: 225–238.

HOLT, G. J., AND C. R. ARNOLD. 1983. Effects of ammonia and nitrite on growth and survival of red drum eggs and larvae. Trans. Am. Fish. Soc. 112: 314–318.

KHOO, K. H., C. H. CULBERSON, AND R. G. BATES. 1977. Thermodynamics of the dissociation of ammonium ion in seawater from 5 to 40°C. J. Solution Chem. 6: 281–290.

KOROLEFF, F. 1976. Determination of ammonia, p. 126–133. *In* K. Grasshoff [ed.] Methods of seawater analysis. Verlag Chemie, Weinheim.

LANG, T., G. PETERS, R. HOFFMANN, AND E. MEYER. 1987. Experimental investigations on the toxicity of ammonia: effects on ventilation frequency, growth, epidermal mucous cells, and gill structure of rainbow trout *Salmo gairdneri*. Dis. Aquat. Org. 3: 159–165.

LEACH, G. J., AND M. H. TAYLOR. 1977. Seasonal measurements of serum glucose and serum cortisol in a natural population of *Fundulus heteroclitus* L. Comp. Biochem. Physiol. 56A: 217–223.

LIDDICOAT, M. I., S. TIBBITTS, AND E. I. BUTLER. 1975. The determination of ammonia in seawater. Limnol. Oceanogr. 20: 131–132.

LLOYD, R., AND L. D. ORR. 1969. The diuretic response by rainbow trout to sub-lethal concentrations of ammonia. Water Res. 3: 335–344.

MATTY, A. J. 1985. Fish endocrinology. Croon Helm, London, 267 p.

MAZEAUD, M. M., AND F. MAZEAUD. 1981. Adrenergic responses to stress in fish, p. 19–75. *In* A. D. Pickering [ed.] Stress and fish. Academic Press, London.

MAZEAUD, M. M., F. MAZEAUD, AND E. M. DONALDSON. 1977. Primary and secondary effects of stress in fish: some new data with a general review. Trans. Am. Fish. Soc. 105: 201–212.

MCFARLAND, W. N. 1959. A study of the effects of anesthetics on the behavior and physiology of fishes. Inst. Mar. Sci. (Port Aransas) 6: 23–55.

PASSINO, D. R. M. 1984. Biochemical indicators of stress in fishes: an overview, p 37–48. *In* V. M. Cairns, P. V. Hodson, and J. O. Nriagu [ed.] Contaminant effects on fishes. Wiley, New York, NY.

PICKFORD, G. E. 1973. The current status of fish endocrine systems. Introductory remarks. Am. Zool. 13: 711–717.

REDNER, B. D., AND R. R. STICKNEY. 1979. Acclimation to ammonia by *Tilapia aurea*. Trans. Am. Fish. Soc 108: 383–388.

SCHRECK, C. B. 1981. Stress and compensation in teleostean fishes: response to social and physical factors, p. 77–82. *In* A. D. Pickering [ed.] Stress and fish. Academic Press, London.

SCHRECKENBACH, K., R. SPANGENBERG, AND S. KRUG. 1975. Cause of gill necrosis. Z. Binnenfisch. DDR 22: 257–288.

SCHULZE-WIEHENBRAUCK, H. 1976. Effects of sublethal ammonia concentrations on metabolism in juvenile rainbow trout (*Salmo gairdneri* Richardson). Ber. Dtsch. Wiss. Komm. Meeresforsch. 24: 234–250.

SHAFFI, S. A. 1980. Ammonia toxicity: metabolic disorder in nine freshwater teleosts. Toxicol. Lett. 6: 349–356.

SMART, G. 1976. The effect of ammonia exposure on gill structure of the rainbow trout (*Salmo gairdneri*). J. Fish Biol. 8: 471–475.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry: the principles and practice of statistics in biological research. 2nd ed. Freeman, New York, NY. 859 p.

SPOTTE, S. 1979. Seawater aquariums: the captive environment. Wiley, New York, NY. 413 p.

SPOTTE, S., G. ADAMS, AND P. M. BUBUCIS. 1984. GP2 Medium is an artificial seawater for culture or maintenance of marine organisms. Zoo Biol. 3: 229–240.

SWIFT, D. J. 1981. Changes in selected blood component concentrations of rainbow trout, *Salmo gairdneri* Richardson, exposed to hypoxia or sublethal concentrations of phenol or ammonia. J. Fish Biol. 19: 45–61.

THURSTON, R. V., R. C. RUSSO, E. L. MEYN, AND R. K. ZAJDEL. 1986. Chronic toxicity of ammonia to fathead minnows. Trans. Am. Fish. Soc. 115: 196–207.

THURSTON, R. V., R. C. RUSSO, AND C. E. SMITH. 1978. Acute toxicity of ammonia and nitrite to cutthroat trout fry. Trans. Am. Fish. Soc. 107: 361–368.

TOMASSO, J. R., K. B. DAVIS, AND B. A. SIMCO. 1981. Plasma corticosteroid dynamics in channel catfish (*Ictalurus punctatus*) exposed to ammonia and nitrite. Can. J. Fish. Aquat. Sci. 38: 1105–1112.

U. S. ENVIRONMENTAL PROTECTION AGENCY. 1985. Ambient water quality criteria for ammonia — 1984. Office of Water Regulations and Standards, Criteria and Standards Division, US EPA, Washington, DC.

VAMOS, R. 1963. Ammonia poisoning in carp. Acta Biol. 9: 291–297.

ZERBE, W. B., AND C. B. TAYLOR. 1953. Sea water temperature and density reduction tables. Spec. Publ. 198, U.S. Coast and Geodetic Survey, Washington, DC. 21 p.

Perivitelline Fluid pH of Rainbow Trout (*Oncorhynchus mykiss*) Eggs in Relation to Ambient pH

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Kügel, B., and R. H. Peterson. 1989. Perivitelline fluid pH of rainbow trout (*Oncorhynchus mykiss*) eggs in relation to ambient pH. Can. J. Fish. Aquat. Sci. 46: 2070–2073.

In soft water of various pH levels, the perivitelline fluid of late eyed rainbow trout (*Oncorhynchus mykiss*) eggs showed significant pH buffering: final perivitelline pH was higher than ambient by 0.7–0.85 pH unit in acidified water but was lower by 0.3–1.0 pH unit in weak acidified or basic water. No significant difference between the pH of an external buffer solution and that of the perivitelline fluid could be observed after 24 h. Most rapid perivitelline pH change occurred in the first 2 h and in the first 10–20 min, when eggs were transferred from acidified to weak acid or basic water and buffer, respectively. Final pH of the perivitelline fluid was reached after 48 h in unbuffered water and 24 h or less in buffer.

Dans les eaux douces à différents niveaux de pH, le liquide périvitellin des oeufs de la truite arc-en-ciel (*Oncorhynchus mykiss*) se révéla comme tampon effectif de pH: le pH périvitelin final était plus élevé (0,7–0,85 pH unit) en eaux acides, mais plus bas (0,3–1,0 pH unit) dans les eaux peu acides ou même alcalines. Aucune différence importante entre le pH de la solution externe et celui du liquide périvitellin ne fut observée après 72 h. Les changements les plus rapides significatifs du pH périvitellin apparurent dans les 2 premières heures et les premiers 10–20 min lorsque les oeufs furent transférés des eaux acides aux eaux et solutions de faibles acidités et alcalines, respectivement. Le pH final du liquide périvitellin fut atteint après 48 h en eau douce et en 24 h ou moins en solution tampon.

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The perivitelline (PV) space between the vitelline membrane and the zona radiata is formed by water uptake (Bogucki 1930; Gray 1932; Peterson and Martin-Robichaud 1982) through the semipermeable zona radiata (Groot and Alderdice 1985; Kügel 1988), as soon as the eggs come in contact with water. While Fisher (1963) considered structural changes of the zona radiata responsible for the water uptake, the osmotic gradient between the PV space and the external medium is usually considered responsible (e.g. Blaxter 1969; Rudy and Potts 1969; Alderdice et al. 1984). The osmotic gradient is established by the osmotically active PV colloid material retained within the zona radiata (Blaxter 1969), probably the zona radiata externa (Groot and Alderdice 1985; Kügel 1988).

Water uptake of teleost eggs lasts until the wall pressure of the elastic zona radiata compensates the hydrostatic pressure (turgor) inside the egg. At that moment the zona radiata becomes completely turgescent and the micropyle is closed. Within salmonid eggs, hydrostatic pressures of 50–60 mm Hg (Alderdice et al. 1984) and osmotic pressures of about 30 mosmol/kg (Rudy and Potts 1969) have been measured.

The perivitelline fluid (PVF) functions as a shock absorber for the developing embryo, as a diffusion barrier which limits embryonal loss of electrolytes (Eddy and Talbot 1985), as an accumulator of cations (especially sodium) (Rudy and Potts 1969; Peterson 1984; Eddy and Talbot 1985), and perhaps as a biochemical buffer.

The decline of fish populations in acidified, poorly buffered freshwater is often caused by disruption of reproduction and development of eggs and fry (Beamish 1976; Harvey 1980; Hoch 1982). Acid shocks during snowmelt periods may coincide with the development of eggs and fry of salmonids (Hagen and Langeland 1973; Grande and Andersen 1979; Lee et al. 1983; Kügel 1984). In general, embryos protected by zona radiata and PVF tolerate lower ambient pH levels than fry (Kwain 1975; Daye and Garside 1977; Johansson et al. 1977; Rombough 1983; Geen et al. 1984; Kügel 1984).

The pH of the PVF affects chorionase activity (thus affecting hatching success) and the physiological well-being of the embryo. Hence, changes in the pH of the PVF in response to the ambient pH and the question of a buffer affect inside the egg was investigated. The final pH and the kinetics of pH change of the PVF after egg transfer to a different pH medium are presented. Such transfers simulate the sudden pH change that occurs when eggs are exposed to poorly buffered waters.

Materials and Methods

Early eyed rainbow trout (*Oncorhynchus mykiss*) eggs were obtained from Rainbow Springs Hatchery, Ontario, and transferred to running tap water at a temperature of 7.5–8.0°C and flow rate of 250 mL/min. The source of water for egg incubation and experiments was Chamcook Lake (N.B.): conduc-

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TABLE 1. pH characteristics of test media for the first series of experiments. Eggs were incubated in all test media 72 h before measurements. Numbers of eggs incubated in each test medium are in parentheses.

pH of water	pH of buffer
8.4 (3)	8.0 (1)
7.3 (6)	7.9 (2)
7.2 (6)	7.8 (2)
7.1 (7)	7.6 (5)
6.8 (4)	6.9 (4)
5.9 (5)	6.3 (5)
5.0 (5)	5.4 (7)
4.2 (5)	4.8 (2)
4.1 (9)	4.65 (3)
3.8 (7)	4.0 (6)
—	3.7 (4)

TABLE 2. Protocols used in measurements of the kinetics of PV-pH change. First column contains pH values of media in which eggs were incubated 72 h prior to transfer. They were then transferred to media of pH levels listed in the second column for time series experiments to determine rates of PV-pH change. Numbers of eggs tested after the various times included in Fig. 2.

Medium	Ambient pH before transfer	Ambient pH after transfer
Tap water	7.0	4.0
	4.0	6.8
Buffer	6.9	3.9
	3.9	6.9

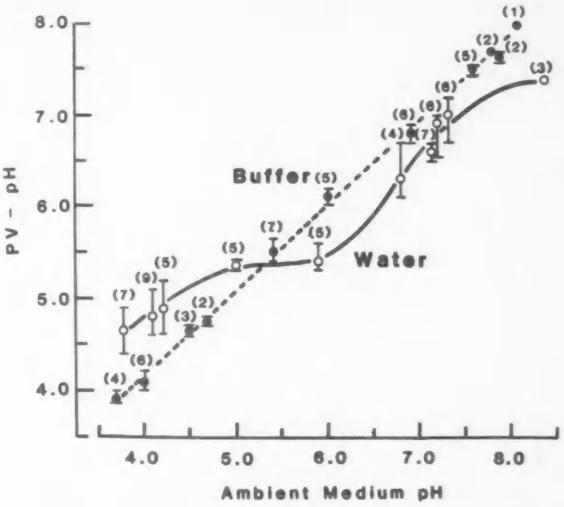

FIG. 1. Final pH of the perivitelline fluid (PV-pH) in response to the pH of tap water (○, solid line) or buffer solution (●, broken line). Number of eggs measured given in parentheses. Bars indicate ranges.

tivity, 47 μS/cm; pH 6.8; Na^+, 2.0 mg/L; K^+, 0.4 mg/L; Mg^{2+}, 0.3 mg/L; Ca^{2+}, 2.3 mg/L; Cl^-, 0.3 mg/L; SO^{2-}, 1.9 mg/L; HCO_3^-, 0.7 mg/L. The water was dechlorinated and filtered before use. Tap water adjusted to various pH levels and prepared buffer solutions served as ambient media in the experiments. The appropriate pH levels (see Tables 1 and 2) were attained by adding dilute H_2SO_4 or NaOH. Tap water was aerated for 2 d after pH adjustment (3.8–8.4) to permit bicarbonate equilibration. Appropriate buffer solutions were prepared using 0.05 M citrate buffer (pH 4.5) and 0.067 M phosphate buffer (KH_2PO_4/Na_2HPO_4, pH 7.4) with pH levels (4.6–7.4) adjusted as described above.

Two series of experiments were performed. In the first the perivitelline pH (PV-pH) was determined after equilibration with the various test media. The eggs were held singly in 2 L of test media at 7–8°C for 72 h before pH measurement. The ambient pH did not change during equilibration (Table 1). In the second series of experiments, information was obtained on the kinetics of PV-pH change after exposure to the test media. Eggs were equilibrated in various ambient media (Table 2, second column) for 72 h and then transferred to the test media and PV-pH measured after 0, 10, 20, 30, 60, 90, 1440, and 2880 min of exposure. Details for various exposures are given in Table 2. The numbers of eggs tested at each test pH for the two experimental series are given in the appropriate figures.

The pH of the PVF was measured with pH microelectrodes imbedded in the tip of a 21-gauge hypodermic needle (Microelectrodes, Inc., USA). Reference electrodes were pulled from filamented borosilicate glass and filled with 3 M KCl, saturated with AgCl. A chloridized silver wire was inserted for connection to the recording apparatus. pH levels were recorded by an Orion model 407 A specific ion meter. The electrodes were moved by micromanipulators.

Each egg was inserted into a hole of slightly smaller diameter in a Plexiglas disc. The disc was in a Plexiglas tray, filled with the corresponding test medium. A water bath maintained the temperature at 7–8°C. The egg was covered by the external medium except for a small dome on the top. This dome was dried with absorbent tissue before being penetrated by micro- and reference electrodes through the same puncture. The microelectrode was inserted near the embryo, where PV space is of greatest depth. Care was taken not to touch embryonal tissue or the vitelline membrane. One egg was used per pH measurement. The electrode system was standardized before and after one to three measurements. The electrodes were rinsed with distilled water before each measurement and dried. In addition, the pH electrode was cleaned periodically with an electrode cleaning solution (Ingold); reference electrodes were replaced several times, as the tips tended to clog with colloidal material.

Results

PV-pH at Equilibrium

Using unbuffered water of various pH values as ambient medium, the final PV-pH is an average of 0.7–0.8 unit above the acidic external pH (pH 3.8, 4.1) and 0.3–1.0 unit lower than the basic medium pH (pH 7.2, 7.3, 8.4) (Fig. 1). In unbuffered tap water adjusted to the lowest pH, the PV-pH is higher than ambient pH up to 5.0. The PVF buffers most effectively between pH 5.0 and 5.9 (Fig. 1).

Whereas the PV-pH usually differed from ambient pH when eggs were reared in tap water of various pH levels, there was almost no difference between PV-pH and ambient pH when buffer solutions were used for incubation (Fig. 1). The PV-pH and the external buffer pH were linearly correlated.

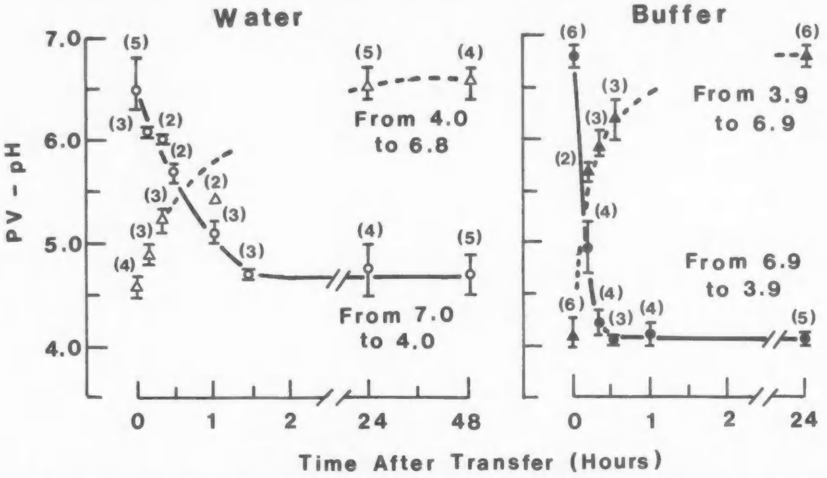

FIG. 2. Time course of the pH of the perivitelline fluid (PV-pH) after egg transfer to differing pH media. Left panel: in water from pH 4.0 to 6.8 (Δ, broken line) and from pH 7.0 to 4.0 (○, solid line); right panel: in buffer from pH 3.9 to 6.9 (▲, broken line) and pH 6.9 to 3.9 (●, solid line). Number of eggs measured given in parentheses. Bars indicate ranges.

To compare the kinetics of change in PV-pH with time after transfer of eggs to media of varying pH levels, the PV-pH was measured at different times after the eggs had been transferred (Fig. 2). The most rapid pH change occurred in the first 2 h, when eggs were exposed to unbuffered water of various pH levels and in the first 10–20 min in buffer solutions. The rate of PV-pH decrease after transfer to acid pH is more rapid than the rate of pH increase after transfer to basic pH. Eggs exposed to buffer of pH 4.0 usually died within 24 h, with few eggs surviving until measurements could be made, while eggs survived 72 h of exposure in unbuffered water of pH 4.0.

Discussion

Buffer solutions and soft water of various pH levels used as incubation media had different influences on the PV-pH of rainbow trout eggs. When eggs were exposed to buffer solutions, no significant difference between ambient and final PV-pH could be seen after 72 h. On the contrary, the PVF showed a significant buffer capacity when eggs were exposed to tap water of various pH: the pH values of the PVF were higher in acidified water but were lower in weakly acidified or basic water. The inflection point was placed between an external pH of 5.0 and 5.9. When Peterson et al. (1980) measured the pH of the PVF in salmon eggs using microelectrodes, they used tap water from the same source as in these experiments. They obtained a final (24-h) PV-pH of 4.4 in water of pH 4.0 and a PV-pH of 5.5 in water of pH 5.5. The value of pH 5.5 matches the inflexion point of the PV-pH curve in experiment 1 closely.

It is probable that the buffering substance in the PVF is not the bicarbonate ion, which has a pK_a of 6.5, as compared with an apparent pK_a near 5.3 for the PVF buffer curve, but that the buffer capacity is provided by the PV colloids. The PV-pH of the eggs of the mollusc *Lymnaea stagnalis* was also found to contain a buffer at low ambient pH (Taylor 1973). The PV buffer capacity is probably the property of the PV colloids, which have a net negative charge at pH levels higher than 4.0 (Peterson et al. 1980). These anionic groups can accept protons and thus provide some buffer capacity in solutions of low ionic strength. Shepard (1986) found no significant difference between PV-pH and ambient pH for media of pH less than 6.7. The ambient media had much higher electrolyte concentrations than the tap water used in these experiments. High ambient cation concentration may neutralize the anionic PV colloids to the extent that they can no longer buffer ambient acidity.

No PV buffering was observed when eggs were exposed to ambient media which were more strongly buffered than the PVF. In this instance the buffering ions (bicarbonate or citrate) from the strongly buffered ambient media enter the PVF, and the buffer characteristics of these ions rather than the PV colloids then determine the PV-pH.

The experiments with buffered ambient media also demonstrated the greater tolerance of encapsulated embryos, compared with newly hatched alevins, when exposed to low ambient pH in poorly buffered waters. This was possibly due to the buffer capacity of the PVF. However, when this buffer system is swamped by a stronger ambient buffer, the emryos die at similar pH levels to those at which newly hatched alevins die (Kügel 1984). It is also significant that the PV-ph buffering range is lower than the bicarbonate buffer range of natural waters, so that the embryo can develop in a reasonably high pH (≥ 4.7) regardless of ambient pH down to about 4.0.

The relatively rapid PV-pH change in response to change in ambient pH reflects the high permeability of the zona radiata and the high mobility of hydrogen ion, as demonstrated in Peterson and Martin-Robichaud (1987).

The pH of the PVF is also relevant when considering chorionase activity at hatching. Hagenmaier (1974a, 1974b found the chorionase activity of rainbow trout eggs to be reduced by 90% at pH 5.2. If the PV-pH falls even lower, hatching success becomes even more difficult due to loss of embryonic physical activity which is required to rupture the outer layer of chorion (Peterson and Martin-Robichaud 1983; Kügel 1988).

These experiments demonstrate the buffering properties of the PVF of the salmon egg in waters of low ionic strength. This property permits the embryo to reside in a milieu of higher pH if ambient pH falls below about 5.3, and thus endows the unhatched embryo greater tolerance to low pH than the hatched alevin. This property, plus the delay in hatching which has been demonstrated at low pH (Peterson et al. 1980), increases the chances for survival for embryos near hatching when low pH events occur in the spring season. Delays in hatching may retain the embryo within the protective PVF for a longer period during temporary pH depressions.

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References

ALDERDICE, D. F., J. O. T. JENSEN, AND F. P. J. VELSEN. 1984. Measurement of hydrostatic pressure in salmonid eggs. Can. J. Zool. 62: 1977–1987.

BEAMISH, R. J. 1976. Acidification of lakes in Canada by acid precipitation and the resulting effects on fishes. Water Air Soil Pollut. 6: 501–514.

BLAXTER, J. H. S. 1969. Development: eggs and larvae, p. 177–252. *In* W. S. Hoar and D. J. Randall [ed.] Fish physiology. Vol. III. Academic Press, NY.

BOGUCKI, M. 1930. Recherches sur la permeabilité des membranes et sur la pression osmotique des oeufs des salmonids. Protoplasma 9: 345–369.

DAYE, P. G., AND E. T. GARSIDE. 1977. Lower lethal levels of pH for embryos and alevins of Atlantic salmon, *Salmo salar* L., continuously exposed to acidic levels of pH, from fertilization. Can. J. Zool. 55: 1504–1508.

EDDY, F. B., AND C. TALBOT. 1985. Sodium balance in eggs and dechorionated embryos of the Atlantic salmon (*Salmo salar* L.) exposed to zinc, aluminum and acid waters. Comp. Biochem. Physiol. 81C: 259–266.

FISHER, K. C. 1963. The formation and properties of the external membrane of the trout egg. Trans. R. Soc. Can. 1 (IV): 323–332.

GEEN, G. H., J. D. NEILSON, AND J. M. BRADFORD. 1984. Effect of low pH on early development of growth and otolith microstructure of chinook salmon, *Oncorhynchus tshawytscha*. Can. J. Zool. 63: 22–27.

GRANDE, M., AND S. ANDERSEN. 1979. Toxicity of acid snowmelt water to brown trout (*Salmo trutta*, L.). Vatten 1: 278–320.

GRAY, J. 1932. The osmotic properties of the eggs of the trout (*Salmo fario*). J. Exp. Biol. 9(3): 277–299.

GROOT, E. P., AND D. F. ALDERDICE. 1985. Fine structure of the external egg membrane of five species of Pacific salmon and steelhead trout. Can. J. Zool. 63: 552–566.

HAGEN, A., AND A. LANGELAND. 1973. Polluted snow in southern Norway and the effect of meltwater on freshwater and aquatic organisms. Environ. Pollut. 5: 45–57.

HAGENMAIER, H. E. 1974a. The hatching process in fish embryos. IV. The enzymological properties of a highly purified enzyme (Chorionase) from the hatching fluid of the rainbow trout (*Salmo gairdneri*, R.). Comp. Biochem. Physiol. 49B: 313–324.

1974b. The hatching process in fish embryos. V. Characterization of the hatching protease (Chorionase) from the perivitelline fluid of rainbow trout, *Salmo gairdneri*, R., as a metalloenzyme. Wilhelm Roux Arch. 175: 157–162.

HARVEY, H. H. 1980. Widespread and diverse changes in the biota of North American lakes and rivers, coincident with acidification. *In* D. Drabloes and A. Tollan. Proc. Int. Conf. Ecol. Impact of Acid Precipitation, Norway, SNSF-Pro9ject, 93–98.

HOCH, J. 1982. Wirkung zweier H^+–Quellen auf frühe Entwicklungsstadien von Forelleneiern. Sportfischer 6: 29–30.

JOHANSSON, N., P. RUNN, AND G. MILBRINK. 1977. Early development of three salmonid species in acidified water. Zoon 5: 127–132.

KÜGEL, B. 1984. Einfluss von Schmelzwasser auf Eintwicklung und Schlupfvorgang bei Regenbogenforellen, *Salmo gairdneri* R. Diplomarbeit, Albert-Ludwigs-Universität Freiburg i. Br., Fakultät für Biologie, West Germany. 77 p.

1988. Untersuchungen zur Entwicklung von Forelleneiern (*Salmo gairdneri*, R., *Salmo trutta, f. fario* L.) in versauierten Gewässern—Investigations on development of trout eggs (*Salmo gairdneri* R., *Salmo trutta f. fario* L.) in acidified water. Ynaug. Dissertation, A.-L. Univ. Freiburg i.Br., West Germany. 142 p.

KWAIN, W. H. 1975. Effects of temperature on development and survival of rainbow trout, *Salmo gairdneri* R., in acid waters. J. Fish. Res. Board Can. 32: 493–497.

LEE, R. M., S. D. GERKING, AND B. JEZIERSKA. 1983. Electrolyte balance and energy mobilization in acid stressed rainbow trout, *Salmo gairdneri*, and their relation to productive success. Environ. Biol. Fishes 8: 115–123.

PETERSON, R. H. 1984. The influence of varying pH and some inorganic cations on the perivitelline potential of Atlantic salmon (*Salmo salar*) eggs. Can. J. Fish. Aquat. Sci. 41: 1066–1069.

PETERSON, R. H., P. G. DAYE, AND J. L. METCALFE. 1980. Inhibition of Atlantic salmon (*Salmo salar*) hatching at low pH. Can. J. fish. Aquat. Sci. 37: 770–774.

PETERSON, R. H., AND D. J. MARTIN-ROBICHAUD. 1982. Water uptake by Atlantic salmon ova as affected by low pH. Trans. Am. Fish. Soc. 11: 772–774.

1983. Embryonic movements of Atlantic salmon as influenced by pH, temperature, and development. Can. J. Fish. Aquat. Sci. 40: 777–782.

1987. Permeability of the isolated Atlantic salmon (*Salmo salar*) chorion to ions as estimated by diffusion potentials. Can. J. Fish. Aquat. Sci. 44: 1635–1639.

ROMBOUGH, P. J. 1983. Effects of low pH on eyed embryos and alevins of Pacific salmon. Can. J. Fish. Aquat. Sci. 40: 1575–1582.

RUDY, P. P. JR., AND W. T. POTTS. 1969. Sodium balance in the eggs of the Atlantic salmon, *Salmo salar*. J. Exp. Biol. 50: 239–246.

SHEPARD, K. L. 1986. The influence of water pH on the perivitelline fluid of perch (*Perca fluviatilis*) eggs. Comp. Biochem. Physiol. 86C(2): 383–386.

TAYLOR, H. H. 1973. The ionic properties of the capsular fluid bathing embryos of *Lymnaea stagnalis* and *Biomphalria sudanica* (Mollusca: Pulmonata). J. Exp. Biol. 59: 543–564.

Comparison of Allozyme and Mitochondrial DNA Variation in Populations of Walleye, *Stizostedion vitreum*

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Ward, R. D., N. Billington, and P. D. N. Hebert. 1989. Comparison of allozyme and mitochondrial DNA variation in populations of walleye, *Stizostedion vitreum*. Can. J. Fish. Aquat. Sci. 46: 2074–2084.

Twelve populations of walleye (*Stizostedion vitreum*) from the Great Lakes and three populations from northern Manitoba were screened for allozyme and mitochondrial DNA (mtDNA) variation. Nine enzyme loci known to show genetic variation were screened in all fish: five of them (*Prot-4, Prot-2, Mdh-3, Idh-1, Adh*) showed appreciable polymorphism. MtDNA was examined in all fish using six endonucleases that detected polymorphic sites and a further 13 endonucleases that detected only monomorphic sites. Only one of the allozyme loci (*Prot-4*) showed evidence of geographic patterning of allele frequencies. By contrast, the mtDNA haplotypes showed clear geographic variation. The proportion of total genetic diversity attributable to population differentiation (*Gst*) was three to five times greater for mtDNA than for the allozymes. *Gst* values for organelle genes are expected on theoretical grounds to be greater than for nuclear genes, and this expected difference may be enhanced in walleye because of the likelihood that, in this species, male-mediated gene flow exceeds that of females. The distributions of mtDNA haplotypes and estimated divergence times are consistent with the derivation of extant walleye populations from three different glacial refugia.

Douze populations de doré (*Stizostedion vitreum*) des Grands lacs et trois populations du nord du Manitoba ont fait l'objet de tests de dépistage des variations d'allozymes et de DNA mitochondrial (DNAmt). Neufs loci d'enzymes dont on sait qu'ils sont sujets à des variations génétiques ont servi à un dépistage chez tous les poissons : cinq d'entre-eux, (*Prot-4, Prot-2, Mdh-3, Idh-1, Adh*) indiquaient un polymorphisme appréciable. Le DNAmt a servi à des tests chez tous les poissons au moyen de six endonucléases qui permettaient d'identifier les sites du polymorphisme, et au moyen de 13 autres endonucléases qui permettaient de détecter seulement des sites de monomorphisme. Un seul de ces loci d'allozymes (*Prot-4*) témoignait d'une répartition géographique des fréquences alléliques. À l'inverse, les haplotypes de DNAmt indiquaient une variation géographique nette. La proportion de la diversité génétique totale attribuable à la différenciation démographique (*Gst*) était trois à cinq fois supérieure dans le cas du DNAmt que dans celui des allozymes. En principe, les valeurs de *Gst* dans le cas des gènes d'organelles devraient être supérieures à ce qu'elles sont pour les gènes nucléaires; cette différence attendue peut être accentuée chez les dorés à cause de la probabilité que dans cette espèce, la circulation génique par les mâles soit supérieure à c elles des femelles. La distribution des haplotypes de DNAmt et les temps évalués de divergence confirment l'origine des populations actuelles de dorés qui proviennent de trois refuges glaciaires.

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The walleye (*Stizostedion vitreum*) is one of the most economically important fish species in the Great Lakes (Regier et al. 1969; Scott and Crossman 1973; Schneider and Leach 1979), and an improved knowledge of its stock structure is essential to a more effective management of this resource. Discrimination of walleye spawning stocks in the Great Lakes has relied almost entirely on tagging studies (Ferguson and Derksen 1971; Wolfert and Van Meter 1978), since morphological and physiological characters (Colby and Nepszy 1981; Riley and Carline 1982) had proven ineffective in stock discrimination and until recently there had been few attempts to look at genetically determined molecular variation.

Published surveys of allozyme variation in walleye have largely been restricted to the analysis of two polymorphic loci: one locus encoding a fast-migrating cytoplasmic malate dehydrogenase and the other a fast-migrating muscle myogen. Clayton et al. (1971, 1974) described three alleles at the *Mdh-3* locus in populations from western Canada, but did not screen fish from the Great Lakes region. The polymorphic myogen was first described by Uthe et al. (1966) in a Lake St. Clair population and subsequently reported in walleye from other Canadian lakes (Uthe and Ryder 1970). In a comparison of three *Stizostedion* species (walleye, sauger (*S. canadense*), and zander (*S. lucioperca*), walleye populations from the Great Lakes region were screened for electrophoretic variants of 39 loci, five of which showed appreciable polymorphism (R. Ward, unpubl. data). Mitochondrial DNA (mtDNA) variation in walleye from this area has been described by Billington and Hebert (1988). Two major groups of mtDNA clones were revealed, an "A" group predominating in the east and a "B" group in the west.

The present paper presents data on the five polymorphic enzyme loci and the mtDNA clones in 12 walleye populations from the Great Lakes region and three populations from Southern Indian Lake, northern Manitoba. A principal aim of this work was to compare the sensitivities of allozymic and mtDNA analyses in revealing genetic differences between stocks. Because organelle genomes are haploid and have uniparental (maternal) transmission, thus contrasting with diploid nuclear DNA with biparental transmission, the effective number of organelle genes is approximately equal to the number of females and is generally only one quarter that of nuclear genes (Nei and Li 1979; Birky et al. 1983). As a consequence, organelle genes will have lower effective migration rates than nuclear genes, and populations will thus tend to be more subdivided for organelle genes than nuclear genes (Takahata and Maruyama 1981; Birky et al. 1983). Only rarely, such as in situations where females greatly outnumber males, is the converse expected (Birky et al. 1989). There have been relatively few direct comparisons of interpopulation divergence for nuclear and mtDNA, although those that have been carried out accord with the above expectations. For instance, mtDNA analysis of the rodents *Geomys pinetis* and *Peromyscus maniculatus* revealed distinct population structuring which was not apparent following allozyme analysis (Avise et al. 1979; Lansman et al. 1983), and the kingcrab *Limulus polyphemus* was shown by mtDNA analysis to be split into northern and southern assemblages whereas allozymes revealed no such clear-cut separation (Saunders et al. 1986). Worldwide populations of *Drosophila melanogaster* showed about three times as much differentiation for mtDNA as for allozymes (Hale and Singh 1987).

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In our study, relative levels of differentiation among populations were assessed using the *Gst* statistic (Nei 1973; Takahata and Palumbi 1985). This parameter reaches equilibrium relatively quickly in comparison with measures such as genetic distance (J. F. Crow, quoted in Takahata and Palumbi 1985) and is insensitive to possible differences in mutation rates (Crow and Aoki 1984). Homing behaviour of walleye to particular spawning streams or local areas has been extensively documented (e.g. Regier et al. 1969; Ferguson and Derksen 1971; Spangler et al. 1977; Bodaly 1980), and thus there are good grounds for expecting significant levels of differentiation between populations of walleye. Unfortunately, published studies have not attempted to differentiate males and females with respect to homing behaviour, although there are substantial unpublished data from fish biologists which indicate that homing behaviour is more marked in females than males (B. Malone, OMNR, Glenora, Ont., pers. comm.; R. Haas, Michigan DNR, Mount Clemens, MI, pers. comm., D. MacClellen, OMNR, Lake St. Clair Fisheries Assessment Unit, Ont., pers. comm.). Increasing philopatry of females is expected to further enhance *Gst* values for mtDNA in comparison with nuclear DNA.

Finally, patterns of allozyme and mtDNA differentiation among populations were examined to see if they lent support to hypotheses concerning the origin of extant walleye populations from glacial refugia.

Methods

Ten populations of walleye were surveyed from within the Great Lakes, two from isolated lakes in the Great Lakes region (Lake Nipissing and Lake Simcoe) and three from the Southern Indian Lake region of northern Manitoba (Table 1).

Nine variable protein loci were screened: alochol dehydrogenase (*Adh*), malate dehydrogenase (*Mdh-3*), isocitrate dehydrogenase (*Idh-1*), creatine kinase (*Ck-2*), adenylate kinase (*Ak*), phosphoglucomutase (*Pgm-1*), phosphoglucose isomerase (*Pgi-2*), and two general proteins (*Prot-4* and *Prot-2*). Two of these loci (*Adh* and *Idh-1*) were screened from liver samples and the remainder from muscle. Separation for all enzymes except malate dehydrogenase was achieved using either horizontal starch gel (12.5% gels, Connaught starch) or cellulose

TABLE 1. Walleye populations analysed. Locations of each site are shown in Fig. 1 and 4. S = spawning populations. The two STC collections were pooled in all analyses, as were the two SIM and ERW collections. The June NIP fish were examined allozymically and the October fish for mtDNA.

Number	Site	Code	Date sampled	Number of fish
1	Muskegon River	MUR	April 1986	33 S
2	Tittabawassee River	TIT	April 1987	12 S
3	Lake St. Clair, off Stoney Point	STC	October 1985	6 —
			October 1986	2 —
4	Thames River	THA	March–April 1985	35 S
5	Western Lake Erie, off Colchester	ERC	October 1985	8 —
6	Chick & Hen Island Reef, Lake Erie	ERW	April 1985	32 S
			April 1986	5 S
7	Lake Simcoe, Talbot River	SIM	April 1986	12 S
	Lake Simcoe, Strawberry Point		October 1987	25 —
8	Trenton River, Bay of Quinte, Lake Ontario	TRE	April 1986	12 S
9	Napanee River, Bay of Quinte, Lake Ontario	NAP	April 1986	12 S
10	Athol Bay, Lake Ontario	ONT	April 1986	12 S
11	Lake Nipissing	NIP	June 1988	8 —
			October 1988	8 —
12	St. Louis River	STL	May 1988	12 S
13	Issett Lake, Man.	IST	July 1987	12 —
14	Southern Indian Lake, Man.	SIL	August 1987	10 —
15	Missi Falls, Southern Indian Lake, Man.	MIF	August 1987	10 —

TABLE 2. Allele frequencies at five variable allozyme loci plus mtDNA clone frequencies in 15 walleye populations.

Locus	Allele/ clone	1 MUR	2 TIT	3 STC	4 THA	5 ERC	6 ERW	7 SIM	8 TRE	9 NAP	10 ONT	11 NIP	12 STL	13 IST	14 SIL	15 MIF
Adh	−60	0.11	0.17	0.08	0.13	0.25	0.20	0.17	0.08	—	0.13	0.13	0.29	0.08	0.20	0.20
	−100	0.89	0.83	0.92	0.87	0.75	0.80	0.83	0.92	1.0	0.88	0.88	0.71	0.92	0.80	0.80
	N	33	12	6	35	8	32	36	12	12	12	8	12	12	10	10
Mdh-3	70	—	—	—	0.01	—	0.03	—	—	—	—	—	—	0.17	0.10	0.15
	100	0.64	0.83	0.50	0.61	0.69	0.75	0.68	0.92	0.63	0.54	0.44	0.42	0.63	0.80	0.55
	120	0.36	0.17	0.50	0.37	0.31	0.22	0.32	0.08	0.38	0.46	0.56	0.58	0.21	0.10	0.30
	N	33	12	6	35	8	32	37	12	12	12	8	12	12	10	10
Idh-1	75	0.21	—	0.08	0.30	0.38	0.27	0.33	0.38	0.37	0.58	0.43	0.50	0.40	—	—
	100	0.79	—	0.92	0.70	0.63	0.73	0.67	0.62	0.63	0.42	0.57	0.50	0.60	—	—
	N	33		6	35	8	32	12	12	12	12	7	12	5	—	—
Prot-4	100	0.80	0.75	0.40	0.53	0.56	0.39	0.58	0.67	0.79	0.71	0.38	0.85	1.0	1.0	1.0
	160	0.20	0.25	0.60	0.47	0.44	0.61	0.42	0.33	0.21	0.29	0.63	0.13	—	—	—
	N	33	12	5	35	8	32	37	12	12	12	8	12	12	10	10
Prot-2	60	0.20	0.13	0.50	0.21	0.31	0.36	0.30	0.71	0.29	0.25	0.50	0.25	0.25	0.13	0.25
	100	0.80	0.88	0.50	0.79	0.69	0.64	0.70	0.29	0.71	0.75	0.50	0.75	0.75	0.88	0.75
	N	33	12	6	35	8	31	33	12	12	12	8	12	6	8	10
mtDNA	1	—	0.17	0.25	0.20	0.43	0.17	0.35	0.50	0.75	1.0	—	—	—	—	—
	2	—	—	—	—	—	0.03	—	—	—	—	—	—	—	—	—
	3	—	—	—	0.02	—	—	—	0.13	0.08	—	—	—	—	—	—
	4	1.0	0.83	0.50	0.63	0.43	0.57	0.54	0.38	0.17	—	0.90	0.27	0.38	—	0.13
	5	—	—	—	—	—	0.07	—	—	—	—	—	—	—	—	—
	6	—	—	0.13	—	—	—	—	—	—	—	—	—	—	—	—
	7	—	—	—	—	0.14	—	—	—	—	—	—	—	—	—	—
	8	—	—	—	0.02	—	0.13	—	—	—	—	0.10	—	—	—	—
	9	—	—	—	0.05	—	0.03	—	—	—	—	—	—	—	—	—
	10	—	—	—	—	—	—	—	—	—	—	—	0.64	0.63	1.0	0.75
	Long	—	—	0.13	0.07	—	—	0.05	—	—	—	—	0.09	—	—	0.13
	Sauger	—	—	—	—	—	—	0.05	—	—	—	—	—	—	—	—
	N	20	12	8	41	7	30	37	8	12	9	10	11	8	10	8

acetate (Titan III gels, Helena Laboratories) electrophoresis, but the MDH isozymes could only be fully resolved on starch. Two buffer systems were employed for the starch system: continuous tris–citrate (electrode buffer 0.25 M Tris, 0.057 M citric acid, pH 8.0; gel buffer 1:25 dilution of electrode buffer; 200 V for 4 h) and discontinuous tris–citrate (electrode buffer 0.30 M borate, pH 8.2; gel buffer 0.076 M Tris, 0.005 M citric acid, pH 8.7; 300 V for 4 h). The latter system was used for alcohol dehydrogenase and the former system for all other allozymes. A single buffer was used for the cellulose acetate gels: tris–glycine (gel and electrode buffers 0.025 M Tris, 0.192 M glycine, pH 8.5; 200 V for 15 min). Stain recipes were modifications of those given by Shaw and Prasad (1970) and Harris and Hopkinson (1976). Alleles are numbered with respect to the mobility of the most frequent allele, designated 100, and loci numbered in order of increasing mobility of their products.

mtDNA methods, interpretations, and a restriction site map are given in Billington and Hebert (1988). All fish were examined using six six-base endonucleases which detected polymorphic sites: *Nco* I, *Sca* I, *Dra* I, *Bst* EII, *Ava* I, and *Cla* I. Smaller numbers of fish, but including samples from each site and also every rare clone, were in addition screened with a further 13 endonucleases which detected only monomorphic cut sites. These enzymes were *Bcl* I, *Eco* RI, *Eco* RV, *Hind* III, *Apa*, *Bam* HI, *Pst* I, *Sal* I, *Sst* II, *Xba* I, *Kpn* I, *Mlu* I, and *Pvu* II.

Levels of population subdivision were estimated using Nei's (1973) Gst statistic. This represents the fraction of genetic variation within the entire sampled area which arises from inter-population genetic differences and is defined as $(Ht-Hs)/Ht$, where Hs is the probability that two genes taken from the same colony are different and Ht is the probability that two genes taken from anywhere in the sampled area are different. For nuclear genes, Hs is equivalent to average sample heterozygosity and Ht to total heterozygosity. Gst is readily estimated for the allozyme data. For mtDNA assayed by restriction enzymes, various methods can be used for estimating Gst. The simplest considers each haplotype as equivalent to an allele and calculates Hs and Ht on this basis. Here, Hs and Ht should be considered as sample and total genetic diversity rather than heterozygosity. A second method is rather more complex and uses site data derived from restriction maps (Takahata and Palumbi 1985). It involves calculating within-population ($\bar{I}$) and between population identities ($\bar{J}$) and deriving Hs as $1-I$ and Ht as $1-((\bar{I}/N)+(1-1/N)\bar{J})$, where N is the number of populations examined.

Genetic distances and relationships among populations were examined in various ways. The allozyme allele frequency data were subjected to two forms of analysis. The first used allele frequencies to derive genetic distances over loci for all pairwise population comparisons (Nei 1972), the resulting matrix being converted into a phenogram using the UPGMA algorithm (Sneath and Sokal 1973). This approach assumes a constant evolutionary rate. The second analysis utilised Felsenstein's (1981) restricted maximum likelihood approach, which operates on the allele frequencies directly rather than through a derived distance matrix, and does not assume constant evolutionary rates. Although this method can be sensitive to the input

TABLE 3. Genetic diversity and subdivision in walleye populations. N = number of populations screened: for explanations of other column headings, see text.

		Great Lakes populations				All populations			
		N	*Ht*	*Hs*	*Gst*	*N*	*Ht*	*Hs*	*Gst*
				(a) Allozyme analyses					
Adh		12	0.247	0.235	0.046	15	0.252	0.241	0.041
Mdh-3		12	0.466	0.423	0.091	15	0.481	0.436	0.093
Idh-1[a]		11	0.454	0.420	0.074	12	0.457	0.425	0.069
Prot-4		12	0.472	0.417	0.116	15	0.424	0.334	0.213
Prot-2		12	0.445	0.397	0.109	15	0.427	0.382	0.106
Mean[b]		12	0.417	0.378	0.094	15	0.408	0.364	0.108
				(b) Mitochondrial DNA analyses					
Haplotypes		12	0.602	0.387	0.357	15	0.672	0.357	0.468
Restriction sites[c]	*a*	12	0.520	0.366	0.295	15	0.504	0.335	0.336
	b	12	0.105	0.069	0.342	15	0.097	0.060	0.382
	c	12	0.049	0.032	0.352	15	0.045	0.027	0.388

[a] Minus population TIT from the Great Lakes and populations MIF and SIL from Southern Indian Lake. These populations were not screened for *Idh-1*.

[b] Mean of the five polymorphic proteins.

[c] Restriction sites: *a*, polymorphic cut sites only ($n = 9$); *b*, all sites cut by the six endonucleases detecting polymorphic sites ($n = 31$, these enzymes cutting in addition at 22 monomorphic sites); *c*, all sites cut by 19 enzymes ($n = 63$, 9 polymorphic and 54 monomorphic sites).

order of populations, we used the "global optimization" routine of the program CONTML (v2.9) which reduces this dependence. CONTML is part of the PHYLIP software package, kindly supplied by J. Felsenstein (Department of Genetics, University of Washington, Seattle, WA). The output is an unrooted tree, but for convenience in display we have arbitrarily chosen to root it at the midpoint of the longest path. The "likelihood distances" shown in our figures are the amounts of expected accumulated variance multiplied by 1000. These two approaches were also used to examine differentiation in mtDNA haplotype frequencies among populations, with the minor change that Rogers' (1972) distance was used in place of that of Nei (1972). This switch arose because some populations had no haplotypes in common, generating a Nei distance of infinity but a Rogers distance of 1. In addition, the mtDNA restriction site maps and site frequencies were used to form a genetic distance matrix of pairwise population comparisons using the approach of Takahata and Palumbi (1985). Here, $D = -\log_e(\bar{J}/\bar{I})$. This matrix was converted into a phenogram using the UPGMA method.

Results

Allozyme Analysis

Allele frequencies (Table 2) were calculated from observed genotype numbers for the five most polymorphic loci (*Adh*, *Mdh-3*, *Idh-1*, *Prot-4*, and *Prot-2*). Of the remaining four loci, single heterozygotes were observed for *Ck-2* (in population NAP), *Ak* (ERW), *Pgm-1*) (ERW), and *Pgi-2* (THA and NIP). These effectively monomorphic loci are not considered further. The *Idh-1* enzyme had degraded and could not be scored in three of the samples (TIT, MIF, and SIL). The three *Mdh-3* alleles, 70, 100, and 120, correspond to Clayton et al.'s (1971, 1974) alleles c1, c2, and c3, respectively. The two *Prot-4* alleles, 100 and 160, correspond to Uthe and Ryder's (1970) A and B alleles, respectively. T. Todd (pers. comm.) has independently screened *Prot-4*, *Adh*, *Idh-1*, *Mdh-3*, *Pgm-1*, and *Pgi-2* from larger samples of a few of the same populations with similar allele frequency results.

All population samples were tested for fits to Hardy–Weinberg equilibrium. A total of 68 tests were carried out, of which three yielded goodness-of-fit probabilities less than 5% (*Adh* in TRE and MIF showed heterozygote deficiencies, and *Mdh-3* in NIP showed a heterozygote excess). This is about the number expected from sampling error and cannot be assumed to reflect real population deviations from equilibrium. However, note that sample sizes were in most cases small, and thus the Hardy–Weinberg test lacks power.

Four of the loci showed statistically significant allele frequency differentiation among populations (*Mdh-3*, pooling the two rarer alleles, $\chi^2_{14} = 29.10$, $P = 0.010$; *Idh-1*, $\chi^2_{11} = 20.58$, $P = 0.038$; *Prot-4*, $\chi^2_{14} = 84.98$, $P < 0.001$; *Prot-2*, $\chi^2_{14} = 38.40$, $P < 0.001$). No significant differentiation was observed for *Adh* ($\chi^2_{14} = 15.37$, $P = 0.35$).

The overall extent of population subdivision was examined in two data sets (Table 3a). One considers gene diversity in the Great Lakes populations alone and the second considers all populations. Four of the allozyme loci, *Mdh-3*, *Idh-1*, *Prot-4*, and *Prot-2*, have very similar total heterozygosity values, but the extent of differentiation between populations varies considerably among them. The fifth locus, *Adh*, shows both reduced heterozygosity and reduced differentiation. Of the four more variable loci, the locus showing the greatest degree of population differentiation is *Prot-4*. This is true when considering only the Great Lakes populations ($Gst = 0.116$) but becomes much more striking when the three collections from Southern Indian Lake are added to the analysis ($Gst = 0.213$). Frequencies of the two alleles in the populations sampled show geographic patterning (Fig. 1), with the *Prot-*

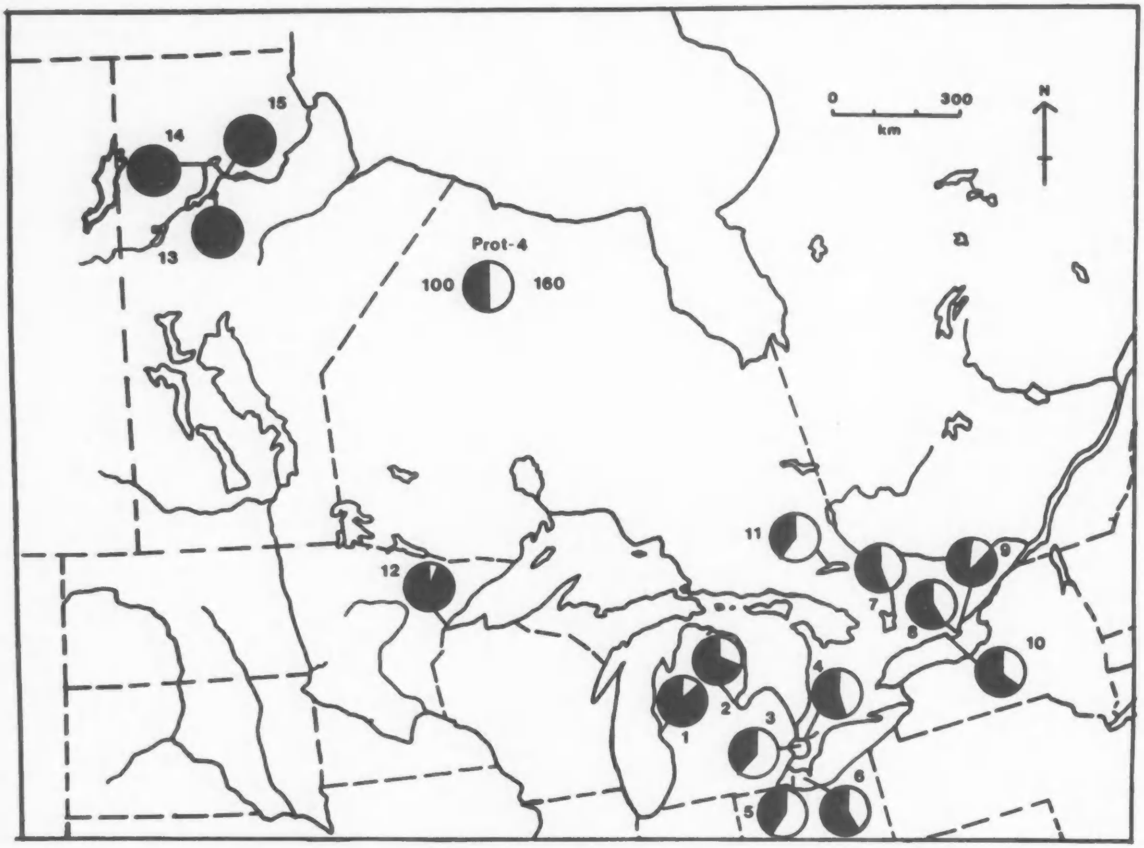


FIG. 1. Relative frequencies of the *Prot-4* 100 and 160 alleles in the 15 walleye populations.

4^{160} allele being most abundant in central populations of the Great Lakes and the *Prot-4*^{100} allele increasing in frequency to the east but more especially to the west, becoming monomorphic in the Southern Indian Lake region. The gene diversity statistics of the other loci are not appreciably affected by the addition of the Southern Indian Lake sites. When considering all populations and loci, $Gst = 0.108$, indicating that about 10% of the total allozymic diversity is attributable to interdeme differentiation.

A UPGMA-derived dendrogram based on Nei's (1972) genetic distance of the 15 populations screened for four allozyme loci (Fig. 2a) shows little evidence of any geographic patterning, other than the grouping together of the three Southern Indian Lake populations. Thus, the apparent patterning of the *Prot-4* locus is masked by the population heterogeneity of the other loci. The TRE sample appears as an outlier, presumably reflecting its relative lack of variation at *Adh* and *Mdh-3* and its aberrant allele frequencies at *Prot-2*. With respect to the latter locus, it is the only sample in which *Prot-2*^{60} is more frequent that *Prot-2*^{100}. Inclusion of the four nearly monomorphic loci *Ck-2*, *Ak*, *Pgm-1*, and *Pgi-2* has no effect on the clusters of Fig. 2*a*.

A second approach used in deriving a phylogeny of these populations was Felsenstein's (1981) restricted maximum likelihood method. Analysis of the 15 populations screened for four polymorphic loci yields essentially two population clusters (Fig. 2*b*), a Southern Indian Lake cluster and a central/eastern Great Lakes cluster. The STL sample occupies an intermediate position, as in fact it does geographically.

Mitochondrial DNA Analysis

Ten different mtDNA clones were identified which could be explained by single site gains or losses. In addition to the nine clones described in Billington and Hebert (1988), a new composite pattern, here identified as clone 10, has been detected. This clone has the following notation (after Billington and Hebert 1988): *Nco* I B, *Sca* I A, *Dra* I A, *Bst* EII A, *Ava* I A, *Cla* I O. Eight walleye (SIM, two fish; THA, three fish; and MIF, STC, and STL, one fish each) showed length variation, having mtDNA molecules between 130 and 1670 base pairs longer than normal. In all cases, this length increase mapped to a region adjacent to the D-loop (Billington and Hebert 1988; N. Billington, unpubl. data). Even when such inserts have similar sizes, they may have independent origins. In addition, two walleye from Lake Simcoe possessed sauger mtDNA (Billington et al. 1988), most probably as a consequence of a past hybridization event. The frequencies of these unusual DNA types are presented in Table 2 (along with clones 1–10) but are excluded from subsequent analyses. Very substantial differentiation in mtDNA clone frequencies was observed, with some populations having no clones in common.

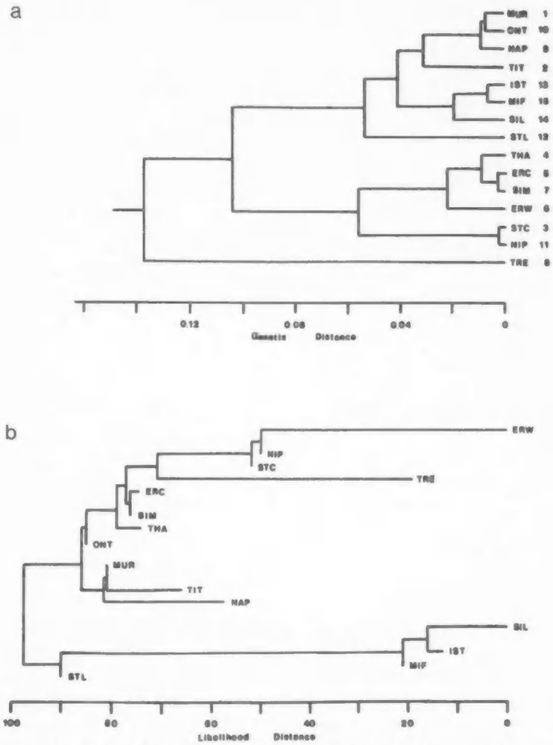


FIG. 2. (a) UPGMA-derived dendrogram of the 15 walleye populations based on genetic distances (Nei 1972) over four polymorphic protein loci. (b) CONTML tree (Felsenstein 1981) based on allele frequencies of the four polymorphic loci, arbitrarily rooted at the midpoint of the longest path.

Clones 1–10 may be linked into a parsimony network (Fig. 3). Billington and Hebert (1988) described two major groups of clones which they designated A and B. The new clone discovered in the present survey, clone 10, is widespread in western walleye populations and in genetic terms is intermediate between clone 2 (a group A member) and clone 4 (a group B member). We categorise this clone as the sole member of a new clonal group, group C. Group A comprises clones 1 and 2, group B comprises clones 4–9, and group C comprises clone 10. Clone 3 occupies a rather anomalous position. It is represented in the parsimony tree by a single site gain from clone 10. However, it is absent from those populations which possess clone 10 and thus may have arisen by two site changes from either clone 1 or clone 4 via an undiscovered intermediate. Both of these clones are frequent in populations with clone 3. The uncertainty concerning the position of clone 3, together with its rarity, has led to its exclusion from the clonal groups A, B, and C.

About 35% of the variation in haplotype frequencies in the Great Lakes populations alone can be attributed to interpopulation variation, a figure which rises to nearly 50% when all populations are considered (Table 3b). When examining mtDNA variation per restriction site rather than per haplotype, these figures are slightly reduced. About 40% of the variation per site can now be attributed to interpopulation variation across the 15 samples.

There is a marked geographic patterning to clone group frequencies (Fig. 4). Clone 10 (group C) is abundant in populations to the north and west of the range sampled, clone group B fish predominate in populations from the central Great Lakes, and group A in the eastern Great Lakes.

A mtDNA UPGMA dendrogram based on Rogers' (1972) genetic distance used in conjunction with haplotype frequencies reveals three major clusters which accord roughly with geographic area (Fig. 5*a*): a western group (MIF, SIL, IST, and STL), a central group (MUR, NIP, THA, ERW, TIT, STC, ERC, SIM, and TRE), and an eastern group (ONT and NAP). The only population to occupy an anomalous position here is TRE: this clusters with the central populations rather than the two geographically adjacent (ONT and NAP).

A phylogenetic tree based on Felsenstein's (1981) restricted maximum likelihood algorithm has been estimated (Fig. 5*b*). Perhaps the most significant difference between this tree and the UPGMA tree is that the three Lake Ontario populations (ONT, TRE, and NAP) cluster together whereas in the UPGMA tree, TRE is in a sister group to ONT and NAP.

Finally, the use of restriction site data generated by the six endonucleases used on all fish (yielding 9 polymorphic and 22 monomorphic sites) enables estimations of within- and between-population identities and distances (Table 4). These give a rather different genetic distance tree (Fig. 6). Here there are essentially two population clusters, one comprising the western sites along with MUR and NIP and the other most of the central and eastern Great Lakes populations. ERW and ONT (central and eastern populations, respectively) fall as outliers to these two groups. This is perhaps surprising considering the apparent similarity of haplotype frequencies between, for example, ERW and THA, and ONT and NAP (Table 2).

Discussion

Uthe and Ryder (1970) and Uthe et al. (1966) screened walleye from five lakes in Canada for *Prot-4* polymorphism. They found that samples from Great Slave Lake and Lake Winnipeg had *Prot-4*^{100} frequencies of 1.00 and 0.94, respectively. Our results are consistent with theirs, as we found fish from Southern Indian Lake (which is about 300 km north of Lake Winnipeg) to be fixed for the *Prot-4*^{100} allele. Both sets of data indicate that populations west of the Great Lakes are fixed, or nearly fixed, for the *Prot-4*^{100} allele, while Great Lakes populations are polymorphic for the *Prot-4*^{100} and *Prot-4*^{160} alleles. Uthe and Ryder (1970) found that samples from Lake Superior, Lake Huron, and Lake St. Clair showed little differentiation (with *Prot-4*^{100} frequencies around 0.45), but our data indicate that the more westerly Great Lakes populations of walleye have increased frequencies of *Prot-4*^{100} (STL, draining into Lake Superior, P = 0.875; MUR, draining into Lake Michigan, P = 0.803).

With respect to *Mdh-3*, the most striking variation resides in the frequencies of the *Mdh-3*^{70} allele. We found this allele to be rare or absent from the 12 Great Lakes populations sampled (weighted average allele frequency, $P = 0.007$) but more common in the Southern Indian Lake populations (P = 0.141). Clayton et al. (1974), in their survey of lakes in southern Manitoba and northwestern Ontario, found that the 70 allele was rare and had a frequency considerably less than 0.10. This generalisation can now be extended to include southern Ontario and the Great Lakes. From a compilation of data from our survey

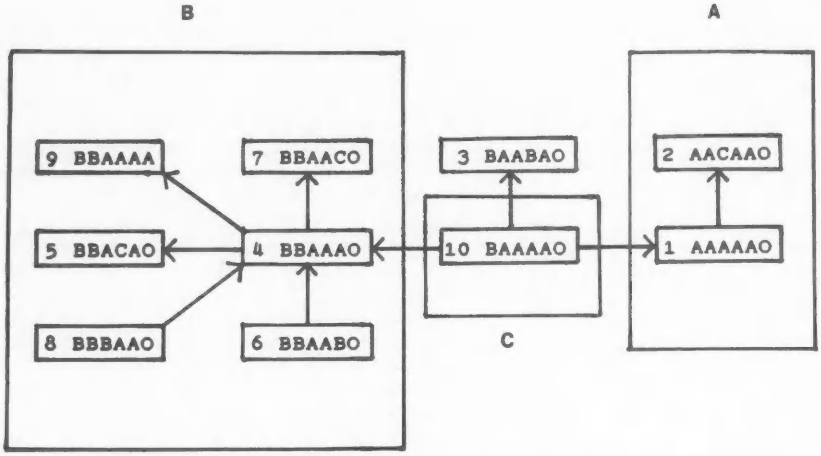
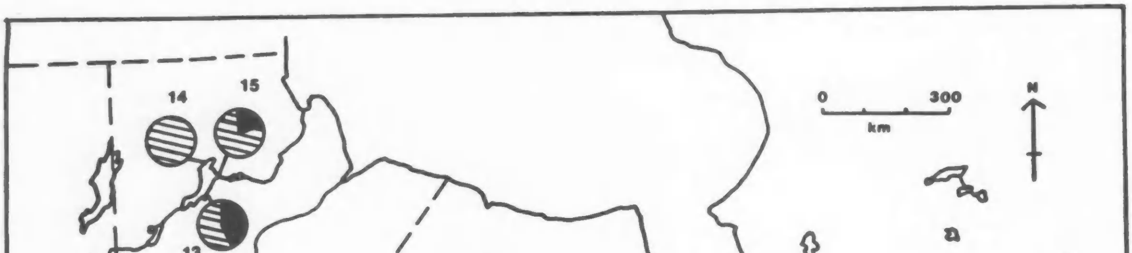

B
A
9 BBAAAA
7 BBAACO
3 BAABAO
2 AACAAO
5 BBACAO
4 BBAAAO
10 BAAAAO
1 AAAAAO
8 BBBAAO
6 BBAABO
C

FIG. 3. Parsimony network of walleye mtDNA clones. Arrows indicate single site gains, not necessarily the direction of evolution. Clones are numbered as in Table 2. The six enzymes considered, with their fragment patterns, are, reading left to right, *Nco* I, *Sca* I, *Dra* I, *Bst* EII, *Ava* I, and *Cla* I. The three major clone groups considered in the text are labelled A, B, and C.


14
15
13
0
300
km
N
C
A
B
11
7
9
8
10
12
2
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4
3
5
6

FIG. 4. Frequencies of the three clonal groups A, B, and C in 15 walleye populations.

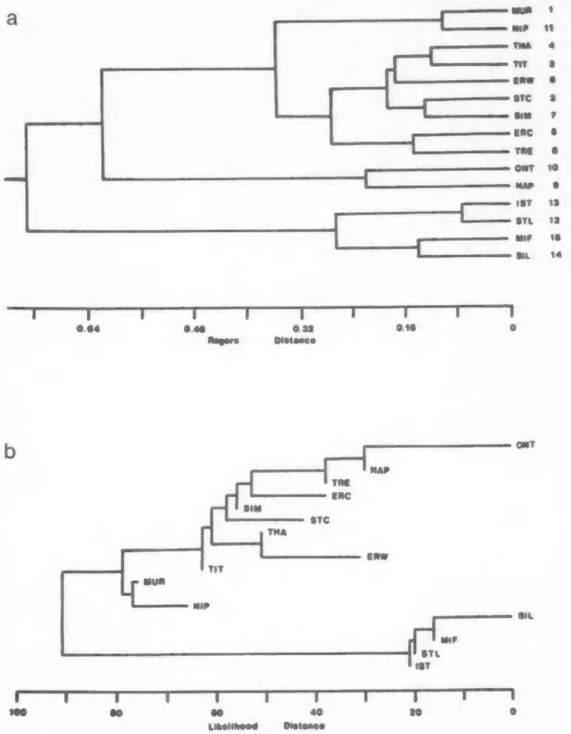

FIG. 5. (a) UPGMA-derived dendrogram of the 15 walleye populations based on mtDNA haplotype genetic distances (Rogers 1972). (b) CONTML tree (Felsenstein 1981) based on mtDNA haplotype frequencies, arbitrarily rooted at the midpoint of the longest path.

and from Clayton et al (1974), it can be seen that the *Mdh-3*^{70} allele is only common in populations from central Saskatchewan, populations in the vicinity of Prince Albert National Park (Central Saskatchewan) having allele frequencies of around 0.60 (Fig. 7). Unfortunately, these populations have not as yet been surveyed for *Prot-4* or mtDNA variation.

The various types of tree produce different population clusters. Perhaps the only uniform feature is that the western populations (SIL, IST, MIF, and STL) always cluster together. It may be argued that the lack of uniformity is a result of the small number of loci or restriction sites examined. For example, the allozyme trees (Fig. 2) use data from only four polymorphic loci. Adding a fifth such locus (*Idh-1*) screened in 12 populations yields very similar clusters. The standard errors of branch points of trees based on only four or five loci are high, but it should be pointed out that it is the polymorphic loci (or restriction sites) alone that will differentiate populations, and adding in monomorphic loci will not affect cluster compositions. We know of no other polymorphic allozymes in walleye, although four further routinely screened loci show single heterozygotes in one or two populations. Another 30 loci appear to be monomorphic (R. Ward, unpubl. data), but these have not been screened in all populations. Other possible reasons for the lack of uniformity may include differences in the assumptions of the clustering methods used and differences in the distribution of nuclear and mtDNA among populations.

In general, the CONTML-derived trees (which do not assume constant evolutionary rates) appear to cluster populations more in accord with their geographic affinities than do the UPGMA trees. For example, the allozyme UPGMA tree locates TRE (from the vicinity of Lake Ontario) as an outgroup to all other populations whereas the CONTML tree clusters it with the other Great Lakes populations (Fig. 2). Similarly, the CONTML mtDNA tree groups together the neighbouring TRE, NAP, and ONT populations whereas the UPGMA tree separates TRE into a sister group (Fig. 5).

While there is significant interpopulation heterogeneity in allele frequency at four of the five polymorphic allozymes screened, and about 10% of allele frequency differentiation can be attributed to interpopulation variation, the extent of mtDNA diversity among populations is three to five times greater (Table 3). As outlined in the introduction, the enhanced ability of mtDNA to reveal population heterogeneity is expected in most species and may be further heightened in the walleye where female-mediated nuclear gene flow is likely to be less than that of males.

TABLE 4. Within-population ($\bar{I}$) and between-population ($\bar{J}$) identity probabilities and genetic distances (D) for mtDNA restriction site data for 15 walleye populations. Values on the main diagonal are $\bar{I}$'s; values above and below the diagonal are $\bar{J}$'s and D's, respectively. Values are calculated using the equations given in Takahata and Palumbi (1985).

		MUR	TIT	STC	THA	ERC	ERW	SIM	TRE	NAP	ONT	NIP	STL	IST	SIL	MIF
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
MUR	1	(1.000)	0.955	0.910	0.884	0.910	0.851	0.946	0.903	0.895	0.923	0.996	0.972	0.975	0.960	0.966
TIT	2	0.020	(0.950)	0.907	0.880	0.909	0.847	0.945	0.904	0.899	0.930	0.951	0.933	0.935	0.923	0.928
STC	3	0.040	0.018	(0.898)	0.843	0.871	0.840	0.904	0.867	0.864	0.894	0.906	0.892	0.894	0.884	0.887
THA	4	0.059	0.037	0.052	(0.877)	0.845	0.817	0.876	0.870	0.867	0.864	0.880	0.864	0.866	0.856	0.860
ERC	5	0.044	0.020	0.034	0.053	(0.905)	0.814	0.908	0.872	0.872	0.905	0.906	0.895	0.897	0.889	0.892
ERW	6	0.076	0.054	0.034	0.051	0.070	(0.842)	0.842	0.809	0.805	0.830	0.848	0.831	0.833	0.823	0.827
SIM	7	0.026	0.001	0.018	0.038	0.017	0.056	(0.943)	0.905	0.904	0.938	0.943	0.930	0.932	0.923	0.926
TRE	8	0.050	0.024	0.037	0.021	0.034	0.074	0.019	(0.901)	0.906	0.907	0.899	0.893	0.894	0.889	0.891
NAP	9	0.064	0.034	0.045	0.031	0.040	0.084	0.025	0.000	(0.910)	0.917	0.891	0.891	0.891	0.889	0.890
ONT	10	0.080	0.047	0.058	0.081	0.050	0.101	0.034	0.045	0.040	(1.000)	0.919	0.923	0.923	0.960	0.923
NIP	11	0.000	0.020	0.040	0.058	0.044	0.075	0.026	0.050	0.064	0.080	(0.992)	0.968	0.971	0.956	0.962
STL	12	0.009	0.025	0.042	0.061	0.042	0.080	0.024	0.042	0.049	0.061	0.010	(0.963)	0.965	0.960	0.962
IST	13	0.007	0.023	0.040	0.060	0.041	0.078	0.023	0.042	0.050	0.062	0.007	−0.001	(0.964)	0.960	0.962
SIL	14	0.041	0.054	0.070	0.090	0.068	0.109	0.051	0.066	0.070	0.041	0.041	0.022	0.023	(1.000)	0.960
MIF	15	0.015	0.029	0.045	0.065	0.044	0.084	0.027	0.043	0.049	0.060	0.015	0.000	0.000	0.020	(0.960)

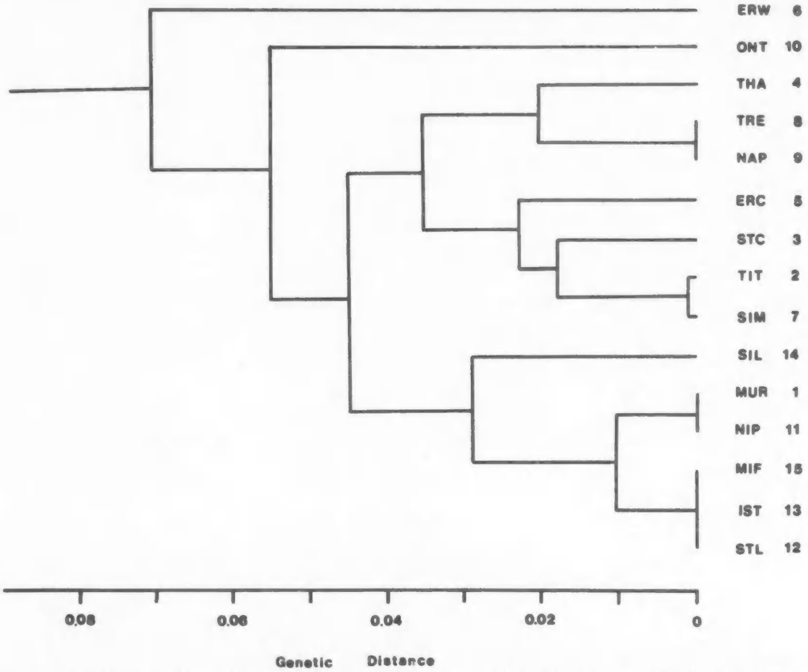

FIG. 6. UPGMA-derived dendrogram of the 15 walleye populations based on genetic distances (Takahata and Palumbi 1985) utilising mtDNA restriction site data and site frequencies.

Variation in mtDNA clonal group frequencies shows clear geographic patterning (Fig. 4). While such patterns may result from genetic drift among populations with limited gene flow, it is interesting to see whether they are consistent with proposals concerning the origins of North American walleye populations. Billington and Hebert (1988) suggested that the degree of genetic divergence between the A and B clonal groups observed in central Great Lakes walleye was consistent with them last having shared a common ancestor about 230 000 (± 100 000) yr ago, and they hypothesised that the two groups reflected colonisation of the Great Lakes from separate glacial refugia. They suggested that group A rose to dominance in populations from an Atlantic refugium, while group B was associated with fish from a Mississippi refugium. This accords with the views of Radforth (1944), who analysed the current distribution of the species in the Great Lakes in relation to time and place of known barriers and connections. Yet the central Great Lakes populations, characterised by the mtDNA B group at high frequency, show little, if any, overall allozyme distinction from those of the eastern Great Lakes, characterised by the mtDNA A group. Any allozyme differentiation arising in these putative refugia appears to have been lost from extant populations, possibly as a consequence of nuclear gene flow exceeding mtDNA flow.

The presence of a third mtDNA group, C, in Southern Indian Lake and western Lake Superior suggests that a third glacial refugium may also have existed. This group is also represented in South Dakota (2 of 13 fish), in Wisconsin fish used to restock the Fox River, Green Bay (one of five), and in Hay River, Northwest Territories (two of five fish) (N. Billington, unpubl. data), giving further support to the possibility of an additional, probably Missourian, refugium. Populations characterised by high frequencies of mtDNA group C (SIL, MIF, IST, STL) also have very high frequencies of *Prot-4*^{100}, which again supports the notion that C group fish derive from a refugium distinct from the B group fish. These populations are well separated from the central and eastern Great Lakes sites, and nuclear gene flow to and from these latter sites will be very low. This will maintain the distinction of the western sites. Crossman and McAllister (1986) noted that sauger used a Missourian refugium in addition to the Mississippi refugium, and suggested that walleye might also have used this refugium. Alternatively, it may be that the B and C groups represent repeated colonizations of walleye from the Mississippi refugium (Bailey and Smith 1981). The group C clone, clone 10, is only separated from the main group B clone, clone 4, by a single restriction site difference (Fig. 3), and thus clonal groups B and C are genetically closer than A and B, which require at least two site changes. Of course, groups A and C are also only one site change apart, but as these show clear geographic differentiation (Fig. 4), it seems reasonable to propose that they arose in distinct refugia. Additional mtDNA and allozyme analyses are being undertaken in order to clarify further some of these incompletely resolved issues.

Acknowledgements

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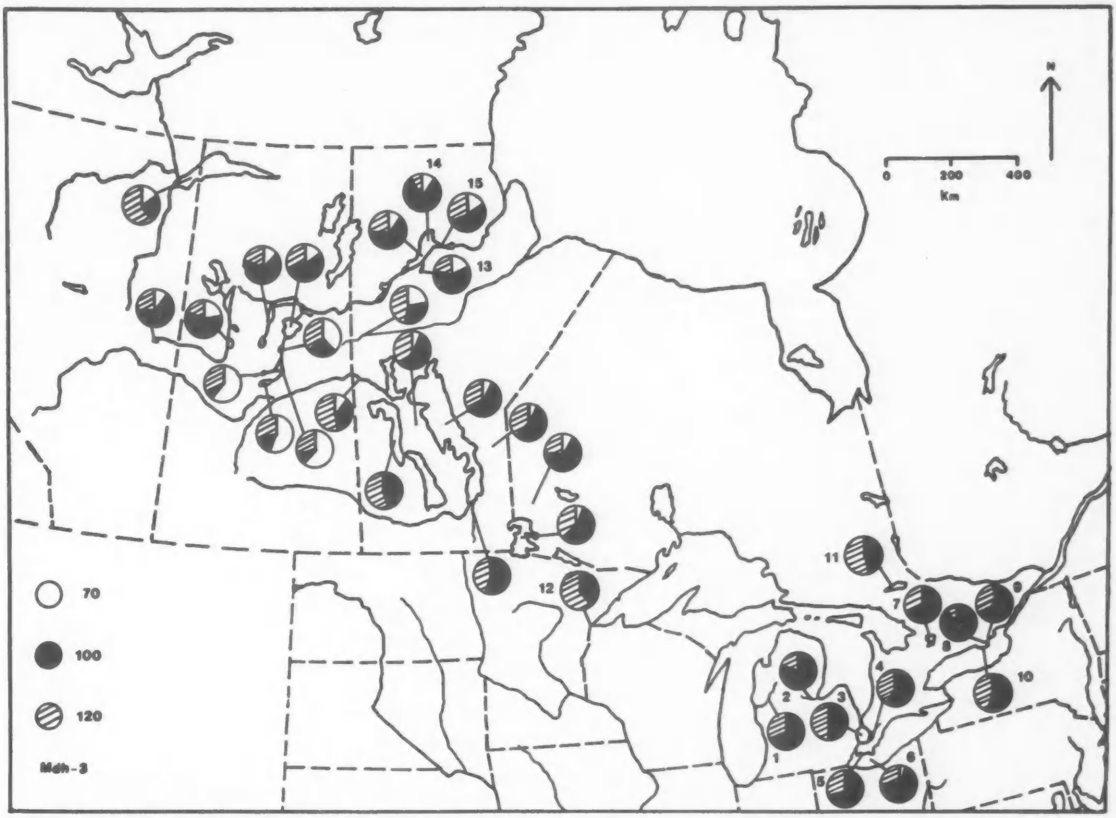

FIG. 7. Allele frequencies of walleye at the *Mdh-3* locus: a compilation of our data and that of Clayton et al. (1974).

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References

AVISE, J. C., C. GIBLIN-DAVIDSON, J. LAERM, J. C. PATTON, AND R. A. LANSMAN. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. Proc. Natl. Acad. Sci. USA 76: 6694–6698.

BAILEY, R. M., AND G. R. SMITH. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. Can. J. Fish. Aquat. Sci. 38: 1539–1561.

BILLINGTON, N., AND P. D. N. HEBERT. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. Aquat. Sci. 45: 643–654.

BILLINGTON, N., P. D. N. HEBERT, AND R. D. WARD. 1988. Evidence of introgressive hybridization in the genus *Stizostedion*: interspecific transfer of mitochondrial DNA between sauger and walleye. Can. J. Fish. Aquat. Sci. 45: 2035–2041.

BIRKY, C. W., P. FUERST, AND T. MARUYAMA. 1989. Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics 121: 613–627.

BIRKY, C. W., T. MARUYAMA, AND P. FUERST. 1983. An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. Genetics 103: 513–527.

BODALY, R. A. 1980. Pre- and post-spawning movements of walleye, *Stizostedion vitreum*, in Southern Indian Lake, Manitoba. Can. Tech. Rep. Fish. Aquat. Sci. 931: 30 p.

CLAYTON, J. W., R. E. K. HARRIS, AND D. N. TRETIAK. 1974. Geographical distribution of alleles for supernatant malate dehydrogenase in walleye (*Stizostedion vitreum vitreum*) populations from western Canada. J. Fish. Res. Board Can. 31: 342–345.

CLAYTON, J. W., D. A. TRETIAK, AND N. H. KOOYMAN. 1971. Genetics of multiple malate dephydrogenase isozymes in skeletal muscle of walleye (*Stizostedion vitreum vitreum*). J. Fish. Res. Board Can. 28: 1005–1008.

COLBY, P. J., AND S. J. NEPSZY. 1981. Variation among stocks of walleye (*Stizostedion vitreum vitreum*): management implications. Can. J. Fish. Aquat. Sci. 38: 1814–1831.

CROSSMAN, E. J., AND D. E. MCALLISTER. 1986. Zoogeography of freshwater fishes of Hudson Bay Drainage, Ungava Bay, and the Arctic Archipelago, p. 53–105. *In* C. H. Hocutt, and E. O. Wiley [ed.] Zoogeography of North American Fishes. Wiley Interscience, New York, NY.

CROW, J. F., AND K. AOKI. 1984. Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. Proc. Natl. Acad. Sci. USA 81: 6073–6077.

FELSENSTEIN, J. 1981. Evolutionary trees from gene frequencies and quantitative characters; finding maximum likelihood estimates. Evolution 35: 1229–1242.

FERGUSON, R. G., AND A. J. DERKSEN. 1971. Migrations of adult and juvenile walleyes (*Stizostedion vitreum vitreum*) in southern Lake Huron, Lake St. Clair, Lake Erie, and connecting waters. J. Fish. Res. Board Can. 28: 1133–1142.

HALE, L. R., AND R. S. SINGH. 1987. Mitochondrial DNA variation and genetic structure in populations of *Drosophila melanogaster*. Mol. Biol. Evol. 4: 622–637.

HARRIS, H., AND D. A. HOPKINSON. 1976. Handbook of enzyme electrophoresis in human genetics. North Holland, Amsterdam, The Netherlands.

LANSMAN, R. A., J. C. AVISE, C. F. AQUADRO, J. F. SHAPIRA, AND S. W. DANIEL. 1983. Extensive genetic variation in mitochondrial DNAs among geographic populations of the deer mouse, *Peromyscus maniculatus*. Evolution 37: 1–16.

NEI, M. 1972. Genetic distance between populations. Am. Nat. 106: 283–292.

1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA 70: 3321–3323.

NEI, M., AND W.-H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76: 5269–5273.

RADFORTH, I. 1944. Some considerations on the distributions of fishes in Ontario. Contrib. R. Ont. Mus. Zool. 25: 116 p.

REGIER, H. A., V. D. APPLEGATE, AND R. A. RYDER. 1969. The ecology and management of the walleye in western Lake Erie. Great Lakes Fish. Comm. Tech. Rep. 15: 101 p.

RILEY, L. M., AND R. F. CARLINE. 1982. Evaluation of scale shape for the identification of walleye stocks from western Lake Erie. Trans. Am. Fish. Soc. 111: 736–741.

ROGERS, J. S. 1972. Measures of genetic similarity and distance. Stud. Genet. VII. Univ. Tex. Publ. 7213: 145–153.

SAUNDERS, N. C., L. G. KESSLER, AND J. C. AVISE. 1986. Genetic variation and geographic differentiation in mitochondrial DNA of the horseshoe crab, *Limulus polyphemus*. Genetics 112: 613–627.

SCHNEIDER, J. M., AND J. H. LEACH. 1979. Walleye stocks in the Great Lakes, 1800–1975; fluctuations and possible causes. Great Lakes Fish. Comm. Tech. Rep. 31: 46 p.

SCOTT, J. B., AND E. J. CROSSMAN. 1973. Freshwater fishes of Canada. Bull. Fish. Res. Board Can. 184: 966 p.

SHAW, C. R., AND R. PRASAD. 1970. Starch gel electrophoresis of enzymes — a compilation of recipes. Biochem. Genet. 4: 297–320.

SNEATH, P. H. A., AND R. R. SOKAL. 1973. Numerical taxonomy. W. H. Freeman, San Francisco, CA.

SPANGLER, G. R., N. R. PAYNE, AND G. K. WINTERTON. 1977. Percids in the Canadian waters of Lake Huron. J. Fish. Res. Board Can. 34: 1839–1848.

TAKAHATA, N., AND T. MARUYAMA. 1981. A mathematical model of extranuclear genes and the genetic variability maintained in a finite population. Genet. Res. 37: 291–301.

TAKAHATA, N., AND S. R. PALUMBI. 1985. Extranuclear differentiation and gene flow in the finite island model. Genetics 109: 441–457.

UTHE, J. F., E. ROBERTS, L. W. CLARKE, AND H. TSUYUKI. 1966. Comparative electropherograms of representatives of the families of Petromyzontidae, Esocidae, Centrarchidae and Percidae. J. Fish. Res. Board Can. 23: 1663–1671.

UTHE, J. F., AND R. A. RYDER. 1970. Regional variation in muscle myogen polymorphism in walleye (*Stizostedion vitreum vitreum*) as related to morphology. J. Fish. Res. Board Can. 27: 923–927.

WOLFERT, D. R., AND H. D. VAN METER. 1978. Movements of walleyes tagged in eastern Lake Erie, N.Y. Fish Game J. 25: 16–22.

Spawning Dynamics of Gulf of St. Lawrence Herring (*Clupea harengus*)

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Mean day of arrival of spawning herring (*Clupea harengus*) in the Gulf of St. Lawrence was calculated for different length groups by region and season. During the spawning season of both spring and autumn groups, herring arrived and deposited eggs on spawning grounds in decreasing order of age and length. Spawning bed surveys at two locations confirmed batch depositions of eggs which were correlated with the presence of dominant age-classes within the spawning population. Due to limitations of the port sampling procedure for collection of biological information routinely used in herring stock assessment, some length groups were underrepresented. This could result in the underestimation of the abundance of recruiting year-classes.

Le jour moyen de l'arrivée de harengs de l'Atlantique (*Clupea harengus*) dans le golfe du Saint-Laurent a été calculé par groupes de différentes longueurs, par région et par saison. Lors de la saison de reproduction des groupes du printemps et des groupes de l'automne, le hareng atteignait les frayères et déposait ses oeufs par ordre décroissant d'âge et de longueur. Des relevés effectués dans les lieux de frai à deux stations ont confirmé le dépôt par lots d'oeufs qui était en corrélation avec la présence de sujets des classes d'âge dominantes dans la population reproductrice. Compte tenu des limites associées à la méthode d'échantillonnage dans les ports, une méthode courante appliquée à l'évaluation des stocks de hareng et qui sert à recueillir des renseignements biologiques, certains groupes de longueur se sont trouvés sous-représentés. Cela pourrait se traduire par une sous-estimation de l'abondance des classes d'âge qui font du recrutement.

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In the Gulf of St. Lawrence a number of studies of Atlantic herring (*Clupea harengus*) have indicated a link between age or size and time of spawning. Jean (1956) noted a decrease in length of herring on spawning grounds at Grande Rivière, Quebec, within both the spring and the autumn spawning seasons. Similarly, Day (1957) reported a decrease in length and age of spring herring during the spawning season in the southern Gulf of St. Lawrence on grounds off New Brunswick and Prince Edward Island. Although mean arrival time at spawning grounds in the southern Gulf seems to be primarily a function of temperature (Messieh 1978; Lambert 1987), it is also related to fish length; larger herring tend to arrive earlier than smaller herring (Lambert 1987). Ware and Henriksen (1978) examined herring landings at spawning grounds in the Southern Gulf of St. Lawrence and noted multiple runs which they reasoned were actually spawning waves, since these runs coincided with the extrapolated origin of larval herring cohorts which subsequently appeared in the plankton. Lambert (1984) confirmed that peaks of landings of herring in St. Georges Bay, Nova Scotia, were synonymous with spawning events.

Recently Lambert (1987) has shown for Norwegian herring that maturation and time of spawning are age specific. This was demonstrated by showing a close correspondence between peaks in egg deposition on spawning grounds and the presence of dominant year-classes within the spawning population. Since time of spawning is age dependent, a population comprising a large number of age-classes would be expected to have a longer spawning season than a population with fewer age-classes. This is significant since, in the Norwegian stock, good recruitment tends to be associated with long spawning seasons and poor recruitment with short spawning seasons (Dragesund 1970). This paper examines data on length- and age-specific time of arrival of mature herring at spawning grounds, combined with information on patterns of egg deposition, to better quantify the spawning behaviour of Gulf of St. Lawrence herring. During this analysis, shortcomings of the port sampling system were discovered. Their effect on the analysis and the implications for stock assessment are discussed.

Data Collection and Methods

Two data sets were used: biological information collected by the National Sampling Programme (NSP) of the Canadian Department of Fisheries and Oceans (DFO) through port sampling of commercial landings, and commercial landing figures collected by the Statistics Division within DFO. Length, age, and maturity data were obtained from routine port samples;

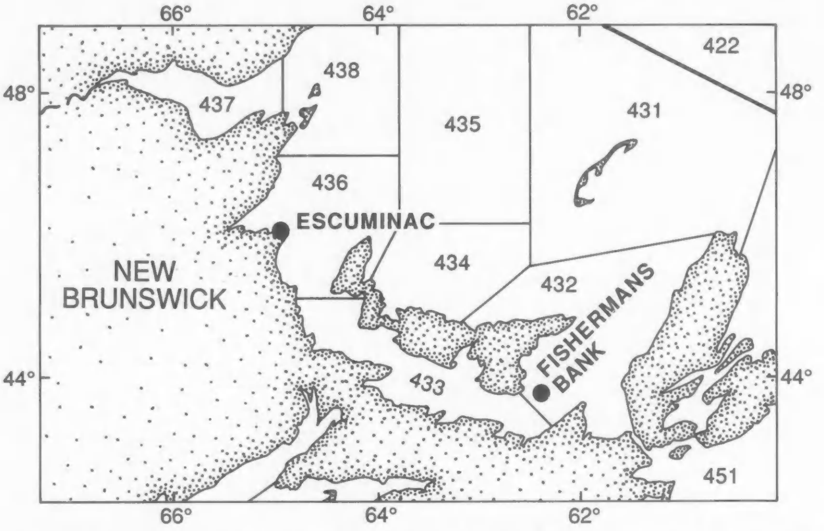

FIG. 1. NAFO statistical areas within subdivision 4T in the Gulf of St. Lawrence showing location of two herring spawning bed surveys (Escuminac and Fishermans Bank).

TABLE 1. Data sets used in analysis; areas in subdivision 4T by year. S = spring, A = autumn; upper case, data used for area analysis; lower case, data inadequate for area analysis but used for subdivision estimates derived from pooled data.

Area	1980	1981	1982	1983	1984	1985
431	S	S	S		s	s
432			A	a	sa	A
433		S	S	sA	a	sa
436	S	A	Sa	sa	a	Sa
437	s	sA	SA	s	sa	sA
438		A	sa	sa	sA	sA

these data were collected in Northwest Atlantic Fisheries Organization (NAFO) subdivision 4T within areas 431, 432, 433, 436, 437, and 438 (Fig. 1). Herring landings were generally sampled during the spawning season at weekly intervals over 4- and 5-wk periods in spring and fall, respectively. This paper uses data for a 6-yr period from 1980 to 1985.

To ensure that catches were representative of a specific area, only those from fixed gillnets and traps were examined. Sampling of catch was fully random except for one case which was stratified (by length) random and thus not included in the analysis. Due to logistical problems stemming from limited resources, the NSP is unable to provide total coverage of all areas. Some areas could not be sampled in consecutive weeks; hence, gaps appear in the data record. A data record for a statistical area that consisted of less than three sampling dates was not used for an analysis of that area, but was used when data for all areas were pooled for an analysis of subdivision 4T (see Table 1).

Although data were available for all maturation stages, only fish in spawning condition, stage VI (Anonymous 1962), were considered. Since the period of sampling was short and confined to just the spawning season, other maturation stages would not have been adequately sampled. For example, the majority of herring would have progressed through stage V (advanced maturity) before the sampling period, but would not have reached stage VII until after.

The progressions of mean length and age over time were calculated from the weekly port samples. Also the mean day of arrival (MDA) at spawning grounds for the different age and length categories was calculated as follows. For each length-group (10-mm interval) or age-group:

$$\mathrm{MDA} = \frac{\Sigma\,(W_i D_i)}{\Sigma\,W_i}$$

where W_i is the total weight of fish in the group in week i and D_i is the midday (Julian) of sample week i and

$$W_i = p_i C_i$$

where p_i is the proportion of the particular length- or age-group in the biological sample in week i and C_i is the total weight of fish in the commercial catch for week i.

Due to difficulty in obtaining commercial landing data for the Magdalen Islands, values of MDA were calculated for that area (431) using port sample numbers alone; that is, W_i in the above equation was the actual number of fish in a particular length category. This was considered adequate, since port sample sizes were large for the Magdalen Islands and presumably they were reasonably representative of landing trends.

The methods employed in the surveying of herring egg beds are described in Messieh et al. (1985). Stage of development of eggs was assessed and time of deposition calculated by back-extrapolation using egg development rates at ambient temperatures (Messieh 1988).

Results

Mean Day of Arrival at Spawning Grounds

Length and age of stage VI herring arriving at spawning grounds decreased over the course of the spawning season in the majority of cases. Mean length and age (mean of length groups and ages weighted by their respective abundances) were

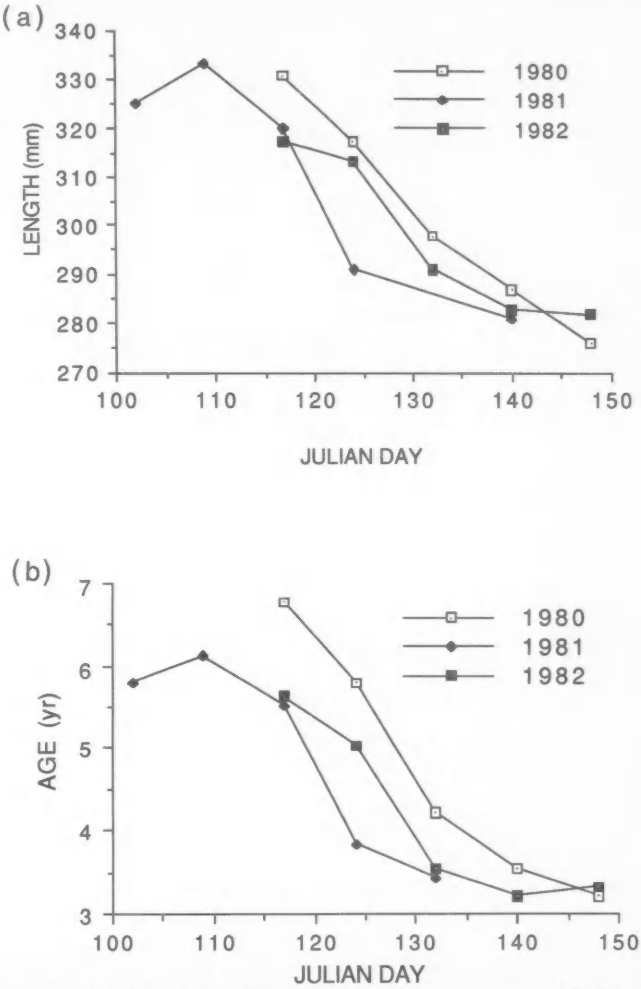

FIG. 2.(a) Mean length (weighted by abundance) and (b) weighted mean age (weighted by abundance) of NAFO subdivision 4T herring (maturity stage VI) during spring spawning season, 1980, 1981, and 1982.

plotted weekly during the spring spawning seasons of 1980–82 as an example (Fig. 2). In general, results were consistent between spring- and autumn-spawning groups of herring, but relationships between age and length and time of spawning were much stronger in the former group. Although herring arrived in decreasing order of size, they did not appear at the spawning grounds in well-segregated groups, as frequency distributions plotted at weekly intervals show considerable overlap (Fig. 3). However, there is more overlap of age-groups than of length-groups; that is, there is a stronger segregation on the basis of size during the spawning season than there is on the basis of age. Assuming the state of maturity of the herring determines its arrival time at the spawning ground, this should not be surprising, for maturation is more a function of size than of age (Motoda and Hirano 1963; Dragesund et al. 1980; Ware 1985). Mainly for this reason, MDA was calculated on the basis of length.

In most cases, MDA, calculated for 10 mm size groups, indicated a progression from large fish (early) to small fish (late), thus demonstrating the same trend of decreasing length as shown in Fig. 2a. There was some curvilinearity in the relationship of size to MDA (Fig. 4) which was not removed by log transformation of the data. Since a simple linear model described the data as well as a logrithmic curve fit, the former was used for simplicity. Although the majority of data collected at the area level yielded inverse relationships between length and apparent spawning time (12 to 19 cases), only 9 cases were statistically significant (Table 2). When all areas were combined, six of nine cases showed an inverse relationship, five of which were significant (Table 3). Probable reasons for the high number of nonsignificant results are discussed later.

Spawning Bed Survey

A spawning bed survey carried out by one of us (Messieh et al. 1985) demonstrated spawning waves in both spring and autumn herring in the southern Gulf (Table 4); the number of discrete egg releases at individual spawning sites ranged from

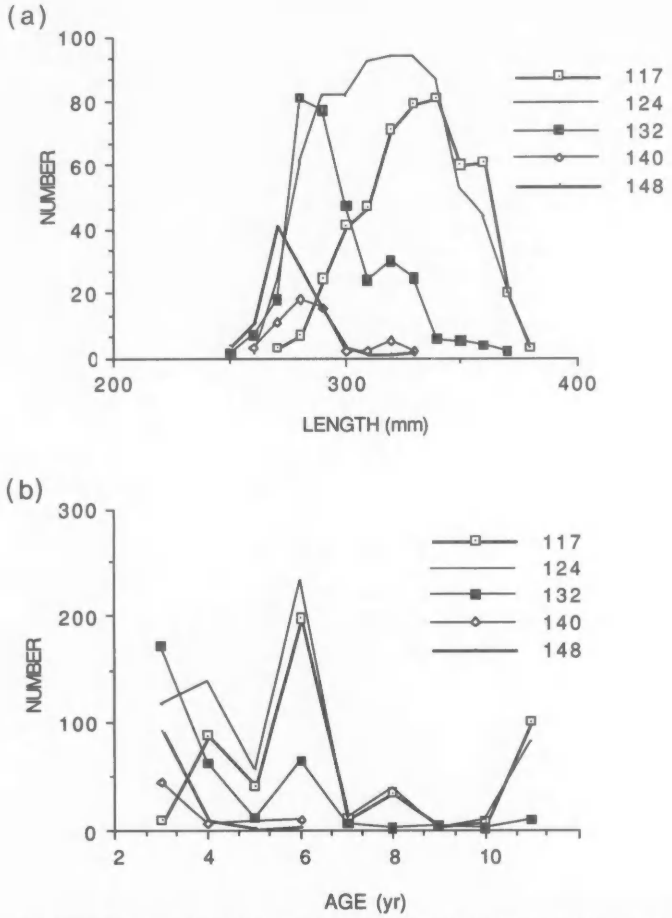

FIG. 3. (a) Length–frequency and (b) age–frequency of NAFO subdivision 4T herring (maturity stage VI) plotted at approximately weekly intervals, spring 1980. Legend numbers are midpoints (Julian day) of successive weeks.

one to four. The pattern of egg release can be matched to length and age composition of the parent population as determined from port sample data in the appropriate areas.

In 1983, at Escuminac (area 436), the spring-spawning population was unimodal, being dominated by 4-yr-old fish (Fig. 5). One spawning wave would be expected, which was the case, as illustrated at the Escuminac spawning grounds on May 20 (Table 4). At the same location, in the spring of 1984, the first egg bed was deposited on May 12. In the next 2 d, two more beds were located but considered the result of "forced spawning" due to intense fishing activity. Such eggs are found in long windrows in line with and in close proximity to gillnets (often underneath) and are often deposited in clumps rather than even layers and on bare rock rather than on red algae which was the more commonly used substrate in this location (Messieh 1988). A final egg bed was deposited on May 27. Sampling of the adult population in the spring of 1984 was inadequate and especially so in area 436. Therefore, samples in adjacent areas were included to construct length- and age-frequency curves (Fig. 6). Although the age–frequency curve is biomodal, the length–frequency curve is trimodal indicating probably three spawning waves. On the basis of the length–frequency curve one would expect one small, early spawning wave of large herring, a second much larger wave of medium-sized herring, and finally a third intermediate-sized wave of small herring. Thus the number and relative size of spawning waves match quite well the number and relative size of length–frequency peaks (Table 5). The fact that the second and largest deposition of spawn was earlier than would have been predicted supports the suggestion of "forced spawning."

Autumn spawning, 1985, on Fishermans Bank (Fig. 1) within area 432 provides another good match (Table 5). The spawning population was trimodal (4-, 7-, and 9-yr age-group peaks) but strongly dominated by 4-yr-olds (Fig. 7). Three spawning waves were documented (Table 4) with the last contributing the majority of eggs, which would be expected, since the youngest fish spawn last.

In general, length- and age-frequency plots for the autumn 1986 population (Fig. 8) sampled in the Fishermans Bank region are not too different from those of 1985 (Fig. 7); however, as Table 4 shows, there was only one spawning event. It appeared that all the year-classes spawned together, since the total egg

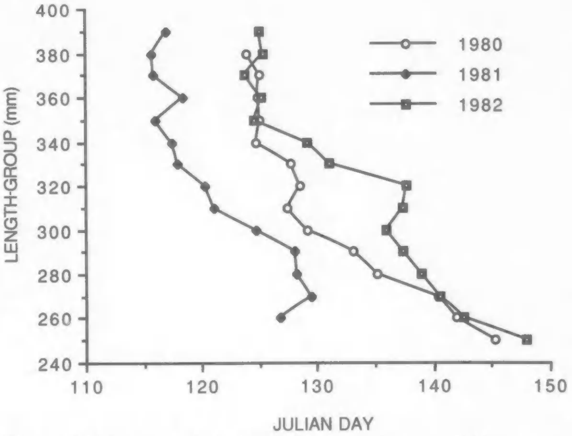

FIG. 4. Weighted mean date of arrival of maturation stage VI at spawning grounds for 4T herring by length-class, spring 1980, 1981, and 1982.

deposition was about the same as for 1985 and 1987. It was brought to our attention by local fishermen that the situation at Fishermans Bank was unusual in 1986. Large quantities of herring in late maturity stage VI ("running" condition with eggs and milt freely flowing when fish subjected to the slightest pressure) were caught at least 2 wk before and until spawning finally occurred. Normally, when such fish arrive at this spawning site, they spawn within a couple of days. However, in this year the herring delayed spawning until some unknown cue initiated the event which we witnessed and identified by an elongated patch of milt approximately 1 naut. mile (1.8 km) wide and 2.5 naut. mile (4.6 km) long. Subsequently, using an underwater television camera, we observed dense layers of eggs on the Bank.

In 1987, multiple spawnings were observed again at Fishermans Bank. At the time of writing, only length–frequency information on herring of unknown maturity stage was available for the area. The distribution (Fig. 9) was bimodal with the right-hand peak being broad and encompassing several older age-classes. It is difficult to match the four spawning events with this length composition, probably because the first three waves occur within 9 d and comprise older fish. Because of slowing growth rates with age, it becomes harder to separate older age-classes on the basis of length, particularly if their numbers are roughly equivalent, as it appears two are in this case, judging by the egg deposition of the middle waves. Also, of course, in this case the length frequency of spawning fish (stage VI) is probably obscured by the inclusion of other maturity stages in the distribution.

The overall match of spawning events to dominant length-classes in the spawning population is summarized as follows:

	No. of length modes	No. of spawning waves
1983	1	1
1984	3	3
1985	3	3
1986	3	1
1987	3?	4

Discussion

It seems surprising that the relationships of length and MDA at the area level are poorer overall than those for the 4T sub-

TABLE 2. Statistical relationship of mean length to mean day of arrival at spawning grounds for spring and autumn herring, maturity stage VI, by area in NAFO subdivision 4T, the southern Gulf of St. Lawrence. $Y = a + bx$ where Y = Julian day and x = mean length (mm). N = number of size intervals ($N - 2$ = degrees of freedom), R^2 = coefficient of determination, P = statistical probability. Length column shows mean seasonal size weighted by abundance.

		Port samples								
Year	Area	Span (wk)	No. of samples	Total no. of fish	Length (mm)	N	a	b	R^2	P
Spring										
1980	431	2	3	1202	324	14	147.8	−0.08	0.77	<0.001
	436	3	4	469	291	11	185.9	−0.18	0.74	<0.001
1981	431	3	4	766	329	13	130.9	−0.05	0.52	<0.01
	433	3	3	292	283	8	149.8	−0.08	0.15	NS
1982	431	3	4	1171	313	13	132.0	−0.02	0.67	<0.001
	433	2	3	425	283	8	119.7	0.08	0.52	0.05
	436	2	3	339	279	8	180.9	−0.14	0.76	<0.01
	437	4	4	101	286	9	113.2	0.07	0.07	NS
1985	436	3	4	152	295	12	118.3	0.04	0.06	NS
Autumn										
1981	436	6	3	272	300	13	211.8	0.11	0.17	NS
	437	4	3	210	292	7	265.6	−0.06	0.21	NS
	438	6	5	331	297	11	251.8	−0.05	0.11	NS
1982	432	3	3	215	309	11	220.7	0.03	0.03	NS
	437	3	3	101	306	9	244.6	−0.08	0.76	0.001
1983	433	3	3	228	314	10	264.8	−0.10	0.67	<0.01
1984	438	6	7	473	318	13	275.8	−0.10	0.62	<0.01
1985	432	3	4	282	320	13	244.0	0.02	0.05	NS
	437	7	3	87	329	10	255.3	0.01	0.01	NS
	438	4	4	126	324	13	290.5	−0.15	0.59	<0.01

TABLE 3. Statistical relationship of mean length to mean day of arrival at spawning grounds for spring and autumn herring, maturity stage VI, in the southern Gulf of St. Lawrence. $Y = a + bx$ where Y = Julian day and x = mean length (mm). N = number of size intervals ($N-2$ = degrees of freedom), R^2 = coefficient of determination, P = statistical probability. Length column shows mean seasonal size weighted by abundance.

	Port samples								
Year	Span (wk)	No. of samples	Total no. of fish	Length (mm)	N	a	b	R^2	P
Spring									
1980	4	5	1730	314	14	180.7	−0.16	0.86	<0.001
1981	5	5	1093	317	14	157.8	−0.11	0.85	<0.001
1982	4	5	2167	298	15	186.2	−0.16	0.92	<0.001
1985	3	4	255	295	11	113.8	0.06	0.13	NS
Autumn									
1981	7	8	764	297	14	201.3	0.12	0.26	NS
1982	4	3	536	309	11	232.2	0.01	0.01	NS
1983	4	4	686	317	13	241.1	−0.01	0.01	NS
1984	7	8	884	318	14	271.3	−0.07	0.52	<0.01
1985	6	7	777	322	15	269.6	−0.06	0.29	<0.05

TABLE 4. Herring egg deposition at two spawning beds in the southern Gulf of St. Lawrence.

		Spawning waves			
Year	Location	Number	Date	Spacing (d)	Number of eggs ($\times 10^{-10}$)
1983	Escuminac	1	May 20		2.2
1984		3	May 12	2	4.8
			May 14[a]	13	12.0
			May 27		9.0
1985	Fishermans Bank	3	Aug. 31	4	86.4
			Sept. 4	12	0.1
			Sept. 16		354.0
1986		1	Sept. 3		418.0
1987		4	Aug. 23	3	44.3
			Aug. 26	6	157.5
			Sept. 1	8	111.0
			Sept. 9		143.0

[a] Forced spawning (see text).

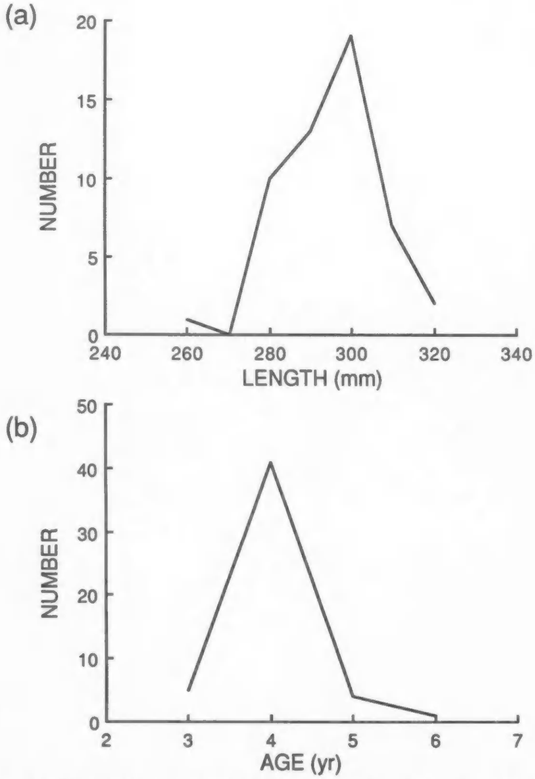

FIG. 5. (a) Length–frequency and (b) age–frequency for stage VI spring-spawning herring in 1983, area 436.

division as a whole. For example, by breaking down total Gulf herring landings to regional units, Ware and Henriksen (1978) were able to identify discrete spawning runs and showed that time of arrival of these runs varied between regions. It was not possible to recognize these spawning patterns within the pooled data set. The reasons for our achieving better results with pooled data than for individual areas treated separately appear to be caused by deficiencies in the port sampling data, specifically in sample size and size-group representation.

First, in cases where inverse linear relationships between length and MDA were statistically significant ($P < 0.05$), the seasonally summed sample size tended to be larger than for those cases which showed nonsignificant relationships. Data for the spring of 1985 (Table 3) are a good example. The total number of fish sampled for this season was 255, much less than for previous years. These fish were collected over four samples, the totals being 32, 78, 21, and 124, respectively. These numbers were distributed among 8–11 length-classes, so it is clear that several of these length-groups were very poorly represented.

Also in the spring of 1985, the four samples were collected over a 3-wk period, a shorter span than in previous years; because of this the data set probably suffered from the second

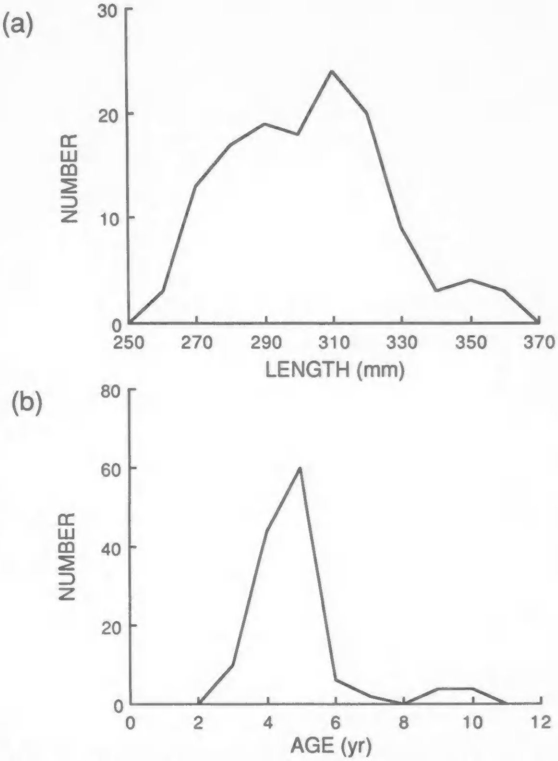

FIG. 6. (a) Length–frequency and (b) age–frequency for stage VI spring-spawning herring in 1984, areas 436, 437, and 438 combined.

and potentially more serious error. When the herring catch is not sampled throughout the entire spawning season, the sample statistics do not give an accurate representation of all the year-classes present in the spawning population. The length of the spawning season can be fairly easily estimated by inspection of peaks in the commercial landings from fixed gear on and near the spawning ground. The port sampling times were superimposed on the landings record and it was clear that many spawning fish were landed at times when there was no biological sampling. Thus, for example, if there were no port sampling in the latter part of the spawning season there would be little or no representation of recruit spawners in the biological data. To test for this possibility, daily commercial landings were totalled for weeks which coincided with biological port sampling, this sum was then expressed as a percentage of the total commercial landings for that particular spawning season and then compared with the number of length-classes (10-mm intervals) represented in the biological samples (Fig. 10). It is evident that when port sampling did not completely cover the spawning season, the number of length-classes was reduced. Lack of complete year-class representation probably contributes to the poor fit of a number of the mean length – MDA relationships (Fig. 11). None of these relationships was statistically significant when port sample representation of the fish landed during the spawning season fell below 57%.

From the point of view of stock management, the impact of underestimating the size of minor year-classes would be small; furthermore, it ought to be possible to recognize this error in more important cases. For example, the problem might be suspected if in one year an age-group that appeared to be small were dominant in the previous year. However, there is, unfortunately, little forewarning of the arrival of new, strong year-classes; thus, lack of sampling at the end of the spawning season could lead to underestimation of recruit spawners which would not be recognized until a later year.

The arrival time of spawners varies between statistical areas; however, the time ''offset'' is small enough that when data for areas are combined, the overall pattern of spawning for the Gulf reflects that of its regions. The limitations of small sample number and inadequate length-class representation are particularly evident in data from statistical areas. However, when the data are pooled these restrictions are reduced and the overall relationship of length to MDA is statistically improved due to the increase in sample size and in degrees of freedom afforded by the wider range of length-classes in the combined data.

For areas where data gave good statistical fits, the MDA of the spawning population was consistent with independant estimates. For example, the MDA of herring (mean length 322 mm) in the Magdalen Islands, area 431, over the period 1980–82 was May 2 ± 6 d (SD) and in area 436, off Escuminac for 1980 and 1982, the MDA of herring (mean length 281 mm) was May 18 ± 6 d (SD). The corresponding values given by Ware and Henriksen (1978) are May 9 ± 6 d (SD) and May 18 ± 7 d (SD) for areas 431 and 436, respectively.

The results of this analysis compare well with those for Norwegian herring (Lambert 1987). In both cases, older herring spawned before younger, and the number of spawning events could be predicted from an inspection of dominant modes in the length–frequency distribution of the mature population. However, whereas time of spawning could be reasonably well predicted for Norwegian herring, the same was not possible for Gulf of St. Lawrence herring in this analysis due to sampling limitations. The Norwegian fishery begins several months before spawning whereas the southern Gulf fishery for the most part occurs immediately before and during the spawning season.

TABLE 5. Predicted amount of herring spawn compared with actual amount of spawn at Escuminac (spring 1984) and Fishermans Bank (autumn 1985). Estimated egg number for largest length mode set equal to actual egg number of first spawning wave. Estimated egg number for other length modes calculated using relative sizes of these modes and correcting for differences in fecundity with length. Fecundities after Messieh (1976). Actual egg number from Table 4.

	Spring 1984			Autumn 1985		
Length modes (mm)	290	310	350	310	340	375
Fecundity/fish ($\times 10^{-3}$)	50	70	110	110	160	225
Relative no. of fish	4	5	1	6	3	1
Predicted egg no. ($\times 10^{-10}$)	8.7	15.3	4.8	253.4	184.0	86.4
Actual egg no. ($\times 10^{-10}$)	9.0	12.0	4.8	253.4	0.13	86.4

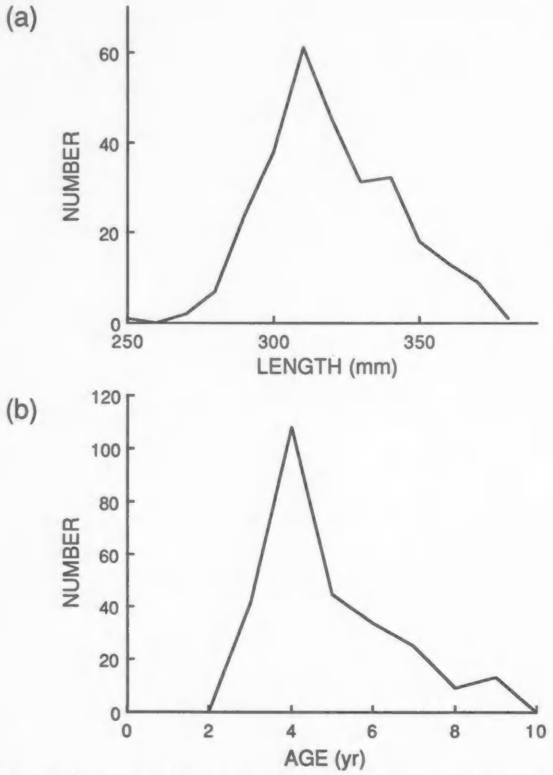

FIG. 7. (a) Length–frequency and (b) age–frequency for stage VI autumn-spawning herring in 1985, area 432.

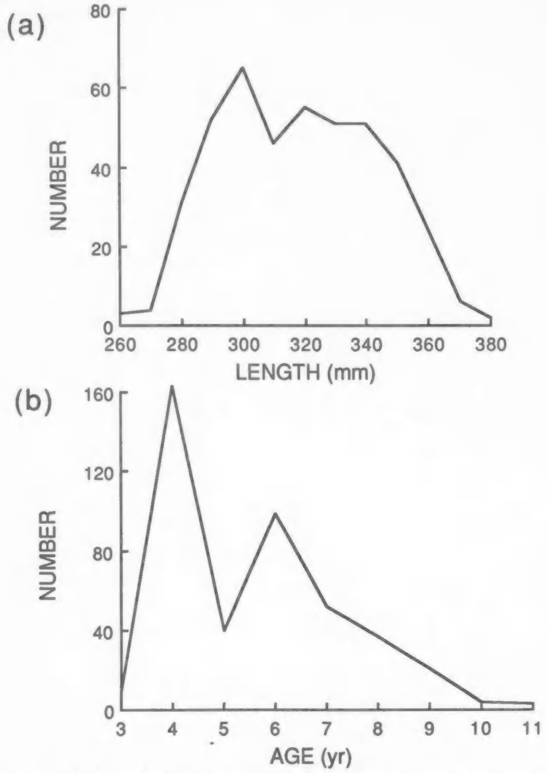

FIG. 8. (a) Length–frequency and (b) age–frequency for stage VI autumn-spawning herring in 1986, area 433.

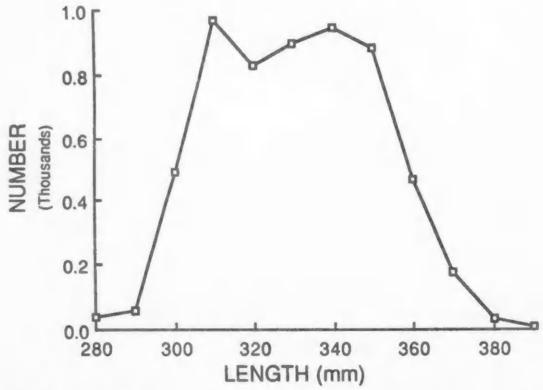

FIG. 9. Length–frequency of stage VI autumn-spawning herring in 1987, area 433.

Accurate estimates of stock biomass are of prime importance to the fisheries manager. A common method used in some form of sequential population analysis (SPA) based largely on commercial landing statistics. However, in the case of herring stocks, such assessments often tend to underestimate declines in biomass and, conversely, overestimate increases (Sinclair et al. 1985). As a result, independent estimates are often sought for corroboration or calibration of SPA estimates. These can be provided by surveys of larvae and of egg deposition at spawning grounds.

Spawning of Atlantic herring is demersal and estimates of spawning stock biomass derived from egg counts are inherently more accurate than those derived from larval counts; however, the latter method is generally preferred, mainly for logistical reasons. On Canada's Pacific coast, where herring spawning occurs intertidally, surveys of egg beds are routine (Humphreys and Hourston 1978; Hourston and Schweigert 1981) whereas on the east coast, underwater survey techniques are required, as spawning is subtidal. Notwithstanding problems of depth, currents, and turbidity, diving surveys, particularly in the southern Gulf of St. Lawrence, are a feasible alternative. In addition to abundance, mortality of eggs can be directly measured at the spawning site, including mass losses associated with storm activity (Tibbo et al. 1963; Lambert 1987; Messieh 1988), losses that preclude the ability of larval surveys to estimate spawning stock biomass reliably. As shown here (e.g. Table 5), the egg survey method of Messieh et al. (1985) seems to describe quite accurately characteristics of the spawning stock. Major spawning sites are well known and tend to be fixed; thus, information on stock structure and a reliable index of stock abundance could be obtained through the monitoring of a few, carefully selected, key spawning grounds.

Acknowledgements

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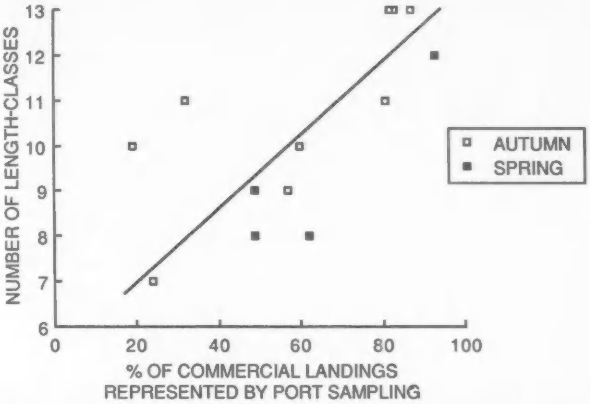

FIG. 10. Relationship of number of length-classes of stage VI herring present in biological samples to the percent of commercial catch, landed during the spawning season, that is represented by this biological sampling. Each point represents one area in subdivision 4T.

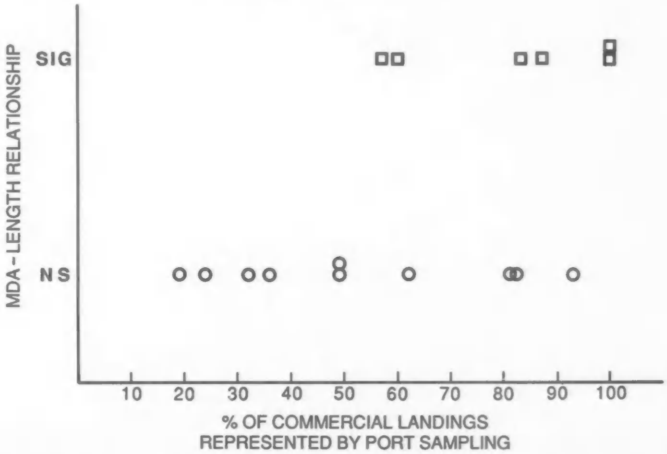

FIG. 11. Percentage of commercial catch, landed during the spawning season, that is represented by biological sampling related to the goodness of fit of the mean length–MDA relationship. SIG (□) are statistically significant and NS (○) are statistically nonsignificant.

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References

ANONYMOUS. 1962. Recommendations adopted by the Herring Committee. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 1: 71–73.

DAY, L. R. 1957. Populations of herring in the southern Gulf of St. Lawrence. Bull. Fish. Res. Board Can. 111: 121–137.

DRAGESUND, O. 1970. Factors influencing year class strength of Norwegian spring spawning herring (*Clupea harengus*). Fisheridir. Skr. Ser. Havunders. 15: 381–450.

DRAGESUND, O., J. HAMRE, AND O. ULLTANG. 1980. Biology and population dynamics of the Norwegian spring spawning herring. Rapp. P.-V. Réun. Cons. Int. Explor. Mer. 177: 43–71.

HOURSTON, A. S., AND J. F. SCHWEIGERT. 1981. Procedures for assessing and forecasting British Columbia herring stocks from catch, sampling and spawn deposition data. Can. Tech. Rep. Fish. Aquat. Sci. 1000: 58 p.

HUMPHREYS, R. D., AND A. S. HOURSTON. 1978. British Columbia herring spawn deposition survey manual. Can. Fish. Mar. Serv. Misc. Spec. Publ. 38: 40 p.

JEAN, Y. 1956. A study of spring and fall spawning herring (*Clupea harengus*) at Grande Riviere, Bay of Chaleur, Quebec. Que. Dep. Fish. Contrib. 49: 1–75.

LAMBERT, T. C. 1984. Larval cohort succession in herring (*Clupea harengus*) and capelin (*Mallotus villosus*). Can. J. Fish. Aquat. Sci. 41: 1552–1564.

1987. Duration and intensity of spawning in herring (*Clupea harengus*) as related to the age structure of the mature population. Mar. Ecol. Prog. Ser. 39: 209–220.

MESSIEH, S. N. 1976. Fecundity studies on Atlantic herring from the southern Gulf of St. Lawrence and along the Nova Scotia coast. Trans. Am. Fish. Soc. 105(3): 384–394.

1978. The decline of the herring fishery in northern Northumberland Strait and its possible causes. CAFSAC Res. Doc. 78/5.

1988. Spawning of Atlantic herring in the Gulf of St. Lawrence. Am. Fish. Soc. Symp. 5: 31–48.

MESSIEH, S. N., R. POTTLE, P. MACPHERSON, AND T. HURLBUT. 1985. Spawning and exploitation of Atlantic herring (*Clupea harengus*) at Escuminac in the southwestern Gulf of St. Lawrence, spring 1983. J. Northwest Atl. Fish. Sci. 6: 125–133.

MOTODA, S., AND Y. HIRANO. 1963. Review of Japanese herring investigations. Rapp. P.-V. Réun. Cons. Int. Explor. Mer. 154: 249–261.

SINCLAIR, M., V. C. ANTHONY, T. D. ILES, AND R. N. O'BOYLE. 1985. Stock assessment problems in Atlantic herring (*Clupea harengus*) in the Northwest Atlantic. Can. J. Fish. Aquat. Sci. 42: 888–898.

TIBBO, S. N., D. J. SCARRATT, AND P. G. MCMULLON. 1963. An investigation of herring (*Clupea harengus*) spawning using free diving techniques. J. Fish. Res. Board Can. 20: 1067–1079.

WARE, D. M. 1985. Life history characteristics, reproductive value, and resilience of Pacific herring. Can. J. Fish. Aquat. Sci. 42 (Suppl. 1): 127–137.

WARE, D. M., AND B. L. HENRIKSEN. 1978. On the dynamics and structure of the southern Gulf of St. Lawrence herring stocks. Fish. Mar. Serv. Tech. Rep. 800: 83 p.

Comparative Vulnerability of Three Esocids to Largemouth Bass (*Micropterus salmoides*) predation

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Wahl, D. H., and R. A. Stein. 1989. Comparative vulnerability of three esocids to largemouth bass (*Micropterus salmoides*) predation. Can. J. Fish. Aquat. Sci. 46: 2095–2103.

We compared vulnerability among tiger muskellunge (*Esox masquinongy* × *E. lucius*) (TM), northern pike (*E. lucius*) (NP), and muskellunge (*E. masquinongy*) (M) to predation by largemouth bass (*Micropterus salmoides*). Equal numbers (about 25/ha) and sizes (either 145, 180, or 205 mm) of each esocid taxa were stocked into three reservoirs (40–89 ha) during 3 yr (five stockings total). Tiger muskellunge were significantly more susceptible to predation ($\bar{x}$ = 30%, range 1–53% mortality) than muskellunge ($\bar{x}$ = 12%, range 2–26%); northern pike were intermediate in susceptibility ($\bar{x}$ = 19%, range 2–35%). Esocid size influenced predation rates for all taxa; losses to predation by largemouth bass decreased from an average of 31% at 145 mm to 2% at 205 mm. Pond experiments (N = 7) provided results similar to reservoirs: TM > NP > M. In laboratory pools with simulated vegetation (N = 106 experiments), susceptibility to predation among esocids did not differ. Dispersal rates by esocids were similar in reservoirs and all taxa preferred vegetated habitats. However, differential habitat selection may partially explain why tiger muskellunge are more vulnerable to largemouth bass predation, as they spent more time in open than vegetated habitats in both pond and pool experiments than either of the parent species. For all taxa, stocking lengths ≥205 mm in fall will increase survival by reducing predatory losses.

Nous avons comparé la sensibilité du Grand Brochet (*Esox lucius*) (NP), du Maskinongé (*E. masquinongy*) (M), et de la forme hybride de ces deux espèces (*E. masquinongy* × *E. lucius*) à la prédation exercée par l'Achigan à grande bouche (*Micropterus salmonides*). Des représentants de même longueur (145, 180, ou 205 mm) de chaque taxon d'Ésocidae ont été maintenus en captivité en nombre égal, soit environ 25 par hectare, dans trois réservoirs d'une superficie de 40 à 89 ha pendant trois ans (cinq empoissonnements au total). La forme hybride était beaucoup plus sensible à la prédation ($\bar{x}$=30 %, mortalité de 1 à 53 %) que le Maskinongé ($\bar{x}$=12 %, mortalité de 2 à 26 %), tandis que le Grand Brochet occupait une position intermédiaire ($\bar{x}$=19 %, mortalité de 2 à 35 %). Chez tous les taxons, la taille influait sur le taux de prédation : les pertes moyennes dues à la prédation par l'Achigan à grande bouche étaient de 31 % chez les sujets de 145 mm de long et seulement de 2 % chez ceux qui avaient 205 mm de long. Des expériences faites dans des étangs (N = 7) ont donné des résultats similaires, à savoir TM>NP>M. Dans des mares artificielles aménagées en laboratoire simulant la présence de végétation (N = 106 expériences), la sensibilité à la prédation était également comparable. Le taux de dispersion des Ésocidae était similaire dans les réservoirs : tous les poissons préféraient les milieux peuplés de végétation. Toutefois, des différences dans le choix de l'habitat peuvent expliquer en partie pourquoi la forme hybride est plus sensible à la prédation exercée par l'Achigan à grande bouche, car celle-ci passait plus de temps dans les milieux ouverts que les représentants des deux autres taxons, que ce soit dans les réservoirs ou dans les mares artificielles. Chez tous les taxons, l'introduction d'individus d'une longueur égale ou supérieure à 205 mm en automne accroîtra le taux de survie par la réduction des pertes dues à la prédation.

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In aquatic systems, predators can determine species diversity and abundance of prey (Hurlbert et al. 1972; Macan 1977; Peckarsky and Dodson 1980; Crowder and Cooper 1982; Morin 1984). From a fisheries management perspective, predators may influence the success of a variety of management practices, including species introductions. However, few studies have examined the importance of predation in determining survival of stocked sport fish.

Predators influence survival of stocked esocids (Weithman and Anderson 1977; Johnson 1978). Predation by largemouth bass (*Micropterus salmoides*) can account for as much as 45% of the mortality of tiger muskellunge (*Esox masquinongy* × *E. lucius*) stocked in reservoirs (Stein et al. 1981; Carline et al. 1986). However, only indirect evidence (Belusz 1978; Johnson 1982; Serns and Andrews 1986) supports the idea that predators control survival of stocked muskellunge (*E. masquinongy*) whereas preliminary evidence (Headrick 1985) suggests that predators do not determine survival of northern pike (*E. lucius*). Although these data suggest the importance of resident predators in determining survival of these three taxa, methodological differences among studies make comparisons of vulnerability impossible. Only by simultaneously stocking similar sizes into the same reservoir can directly comparable data for tiger muskellunge, muskellunge, and northern pike be obtained.

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Esocids are often artifically propagated and differences in hatchery rearing conditions may contribute to behavioral differences among taxa making them more or less vulnerable to resident fish predators. Reared in intensive culture with less cannibalism than northern pike, tiger muskellunge more readily accept a dry diet than muskellunge (Graff 1978). As a result, tiger muskellunge are fed pellets in hatchery troughs whereas northern pike and muskellunge are generally grown to stockable sizes on minnows in hatchery ponds. These differences in rearing environment may affect esocid habitat selection and susceptibility to predation. Habitat use influences risk to predation in other taxa (Werner et al. 1983; Kotler 1984; Mittelbach 1984) and we hypothesized that tiger muskellunge may be more susceptible than its parents to predation. In this study, we compare risk to largemouth bass predation among muskellunge, northern pike, and tiger muskellunge in laboratory, pond, and field experiments. With these data, we quantify differential habitat selection among esocid taxa and evaluate its influence on relative susceptibility to predation.

Materials and Methods

Field Experiments

To compare susceptibility to largemouth bass predation among tiger muskellunge, muskellunge, and northern pike, we stocked equal numbers and sizes (either 145, 180, 205 mm) of esocids into three Ohio reservoirs during 3 yr (Table 1). All esocids were reared at Ohio Division of Wildlife fish hatcheries. Tiger muskellunge were reared in troughs and fed a dry pellet diet whereas muskellunge and northern pike were raised on fathead minnows (*Pimephales promelas*) in ponds. To control growth and match final sizes, muskellunge and northern pike were transferred to troughs for 1 mo before stocking. Esocids were counted and a sample ($N = 100$) measured and weighed the day before each stocking (Table 1). Fish were transported to the reservoirs in aerated tanks with 0.5% salt (see Mather and Wahl (1989) for details of transport conditions). To evaluate poststocking mortality, we held 30 of each taxon in floating cages (2.6 × 0.9 × 0.9 m) for 4 d; low (<6.6%) nonpredatory mortality occurs immediately after stocking (Mather and Wahl 1989).

To estimate losses of esocids to predation, we examined stomachs from largemouth bass captured by electroshocking equally spaced transects (seven in North Reservoir and five each in Madison and Kokosing reservoirs) along the entire perimeter of the reservoir on days 1, 2, 3, 5, and 7 poststocking and at weekly intervals thereafter for 60 d (range = 30–113 largemouth bass examined per sample date). Largemouth bass were measured (nearest 1 mm), weighed (nearest 1 g), fin-clipped, stomach contents recovered by pulsed gastric lavage (Foster 1977), and released. In the laboratory, prey obtained from largemouth bass stomachs were identified; northern pike and muskellunge were differentiated by vertebral structure (Crossman and Casselman 1969). Esocids from our stockings could not be confused with resident young-of-year fish, as no natural reproduction occurs in any of the three reservoirs. Tiger muskellunge, which have vertebrae similar to northern pike, were marked before stocking with oxytetracycline (Wahl and Stein 1987). Vertebrae of esocids recovered from largemouth bass stomachs were examined under ultraviolet light and tiger muskellunge identified by the presence of a yellow-gold flourophor. Esocids recovered from largemouth bass stomachs were measured as total, standard, or backbone length depending on stage of digestion (nearest 1.0 mm). Total length of each esocid was estimated by regression equations between total lengths and either standard or backbone lengths ($N = 50$ individuals/taxon) (see Fig. 1).

Abundance of largemouth bass was estimated using Schnabel mark–recapture procedures (Ricker 1975). Total densities of 1+ and older largemouth bass were similar among the three reservoirs (range = 31–39/ha). Size frequency distributions of largemouth bass also were similar among reservoirs and years (Kolmogorov–Smirnov test, $p > 0.05$), except for Kokosing Reservoir which had higher numbers of 200- to 235-mm fish. Even though density and size distribution of largemouth bass were similar among reservoirs, the proportion of largemouth bass capable of consuming esocids varied with the sizes stocked. As a result, the functional density of largemouth bass was lower for stockings of large as compared with small esocids. To avoid overestimating losses of esocids to predation, we included only largemouth bass capable of ingesting esocids in our density estimates (see Table 2). Minimum lengths of largemouth bass included in each population estimate were determined by dividing the length of the smallest stocked esocid by the largest prey/predator length ratio computed from lengths of esocids recovered from largemouth bass stomachs (as per Carline et al. 1986). No differences in prey/predator length frequencies occurred among esocid taxa (Fig. 1); regressions of cumulative frequency against prey/predator length were similar (ANCOVA, homogenity of slope, $p > 0.14$). As a result, we used the largest prey/predator length ratio (0.75) computed from combined data to determine the minimum length of largemouth bass to include in each population estimate; minimum lengths varied among reservoirs depending upon esocid stocking size (Table 2). Estimates of mortality by predation (Y) for each taxon (i) were determined as

$$Y_i = \sum_{j=1}^{d} a_j / b_j \cdot x$$

where a = the number of each esocid taxa recovered from

TABLE 1. Summary of field conditions associated with experiments comparing susceptability to largemouth bass predation among tiger muskellunge (TM), northern pike (NP), and muskellunge (M). Length (nearest 1.0 mm) and weight (nearest 0.1 g) for each taxon were based on a sample ($N = 100$) measured the day before stocking.

Reservoir (ha)	Stocking date	No. of each taxon	No./ha	Total length (95% C.I.)			Wet weight (95% C.I.)		
				TM	NP	M	TM	NP	M
North (89)	29 Aug. 1983	1150	13	183 (±3.0)	—	180 (±3.9)	29.0 (±1.6)	—	24.6 (±1.9)
Kokosing (46)	9 Aug. 1984	1150	25	145 (±2.4)	145 (±2.5)	147 (±4.5)	12.8 (±0.7)	11.2 (±0.8)	11.4 (±1.3)
North	4 Sept. 1984	3000	34	177 (±2.2)	179 (±3.5)	179 (±4.2)	24.4 (±1.1)	30.7 (±1.9)	23.9 (±1.9)
Madison (40)	29 July 1985	1000	25	147 (±2.8)	143 (±1.7)	144 (±3.0)	13.9 (±1.0)	14.7 (±0.8)	11.8 (±1.0)
North	26 Aug. 1985	2150	24	208 (±4.5)	205 (±4.7)	205 (±3.9)	44.3 (±2.7)	44.6 (±3.6)	39.4 (±2.6)

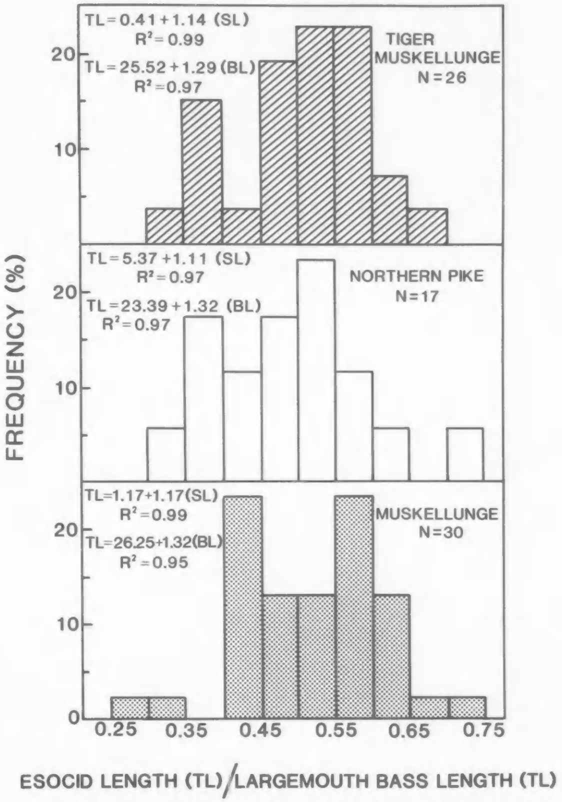

FIG. 1. Frequency distribution of the total length of each of the three esocids recovered from stomachs divided by the total length of the largemouth bass that consumed them. Regression equations, used to estimate total length (TL) from partially digested standard lengths (SL) and backbone lengths (BL), are presented for each esocid taxon.

largemouth bass stomachs during a sample (j), b = number of bass stomachs examined during a sample, x = number of largemouth bass, and d = number of days following stocking (60 in our experiments). Values between sampling dates were estimated by linear extrapolation. These relative estimates of esocid losses to predation assume largemouth bass stomach contents represent 1 d of feeding. At water temperatures encountered during our study (20–22°C), evacuation rates of 21–26 h appear reasonable for largemouth bass (Beamish 1972; Hunt 1960), supporting our assumption.

To explain differential susceptibility to predation among esocids, habitat selection and taxon-specific dispersal patterns following stocking were quantified in North Reservoir. Of the seven equal length transects into which we divided North Reservoir, the two farthest from the single stocking site contained dense stands (75% of shoreline) of lily pads (*Nymphaea* spp.) whereas the other transects were without vegetation. The proportion of esocids captured in these two transects allowed us to assess both differential habitat selection and dispersal rates.

Pond Experiments

Relative vulnerability of the three esocids to largemouth bass predation also was estimated in pond experiments conducted during 3 yr (N = 7 experiments). Ponds were uniform in size (0.05 ha, 1.5 m deep) and were clay bottomed; at pond edges, terrestrial vegetation (grasses) extended 1 m from the perimeter. Experimental design differed among years. In 1983, we stocked each of three ponds with 10 largemouth bass (250–300 mm) collected from Ohio reservoirs. One week after largemouth bass were stocked, we added 90 individuals of tiger muskellunge or muskellunge or northern pike of similar size to each pond. In 1984 and 1985, we divided each of six ponds into two equal sections with netting. Six largemouth bass, collected either from reservoirs (1984) or hatchery ponds (1985), were assigned randomly to one half of each pond. Both halves were stocked with 50 individuals of a single esocid taxon, each marked uniquely by the removal of a left or right pelvic fin. After 9 d, ponds were drained to recover remaining esocids. To directly estimate losses of esocids to predation, we seined largemouth bass three or four times during each experiment and pumped their stomachs.

Habitat selection by the three esocids was assessed by snorkel observations. Three transects, two along the pond edge and one through the center, were swum on days when ponds were seined. Habitat (open or vegetated, upper or lower half of water column) was quantified for all esocids sighted.

Laboratory Experiments

To evaluate the effect of vegetation on susceptibility to predation among the three esocids, we conducted 106 experiments in circular pools (2.5 m in diameter, 0.5 m deep) at water temperatures of 18–23°C and a photoperiod of 14 h light : 10 h dark. Half the pool contained simulated aquatic vegetation (250 stems/m^2) made from polypropylene line (0.5 m long, 4 mm

TABLE 2. Schnabel population estimates of largemouth bass in North, Kokosing, and Madison reservoirs determined following esocid stockings. Lengths of largemouth bass included in population estimates were based on the minimum size capable of ingesting the smallest esocid stocked. The 95% confidence intervals were calculated from fiducial limits for the binomial distribution (Ricker 1975).

Reservoir (year)	Minimum total length (mm) marked	Population estimates			
		Point estimate	95% C.I.	No./ha	% marked
North (1983)	210	1134	991–1324	13	47
Kokosing (1984)	170	1758	1349–2520	38	20
North (1984)	200	1860	1645–2138	21	23
Madison (1985)	170	930	821–1072	23	52
North (1985)	235	1030	782–1509	12	25

in diameter) whereas the other half contained no "vegetation." Line sections were fastened to wire mesh, covered with gravel, and extended to the water surface. These simulated vegetation densities are within the range of values observed for natural systems (Savino and Stein 1982).

Groups of six naive, similar-sized esocids (140–210 mm, 50–60% of largemouth bass length), two of each species, were added to the pool 24 h before an experiment. Preliminary experiments (N = 15/taxon), with two individuals of a single taxon, indicated that habitat selection (N = 4 tank locations) among the three esocids was not influenced by the presence of the other taxon (MANOVA, $p > 0.15$). Esocid location (upper or lower 0.25 m of water column) and habitat selection (open or vegetated portion of pool) were recorded for 1 h before and after introduction of the largemouth bass. Esocid habitat selection was recorded every 2 min (N = 30 observations/experiment); mean values for each experiment were compared among esocid taxa. Largemouth bass (250–350 mm) starved for 24 h were released into the pool from a holding compartment. We used 15 largemouth bass; each individual was used in five to nine experiments. We attempted to evaluate largemouth bass behavior while capturing esocids, but capture rates of prey were extremely low (about one esocid per day). Following release of the largemouth bass, we quantified flight distances for each esocid and recorded habitat selection and initial distance from the largemouth bass for esocids followed. From these data, we compared behavior of esocids followed with those not followed by the largemouth bass. After 24 h we counted remaining esocids.

Results

Field Experiments

We recovered esocids from largemouth bass stomachs following each reservoir experiment (Table 3). All consumption of esocids by largemouth bass occurred within 30 d following stocking; most losses occurred during the first 7 d. Based on estimates of the number of largemouth bass capable of ingesting esocids (Table 2) and daily estimates of the number of each esocid taxon eaten by this predator, we estimated that substantial numbers (range = 2–53%) of all three esocid taxa stocked were eaten by largemouth bass (Table 3). Tiger muskellunge ($\bar{x}$ = 30%, range 1–53%) were more vulnerable to predation than muskellunge ($\bar{x}$ = 12%, 2–26%) (ANOVA, $p < 0.05$, Tukey's multiple comparisons, $p = 0.05$) whereas northern pike ($\bar{x}$ = 19%, 2–35%) were intermediate (Fig. 2). Losses of esocids to predation were negatively related to size for tiger muskellunge (Pearson correlation, $r = -0.92, p = 0.03$) and northern pike ($r = -0.95, p = 0.03$), but not for muskellunge ($r = -0.44$, $p = 0.46$). For all three taxa, predatory losses were least for the largest size class stocked (205 mm).

Abundance of alternate prey for largemouth bass could influence predation intensity on esocids. Because traditional sampling gears underestimate the abundance of gizzard shad, the primary forage in these systems, we used the proportion of largemouth bass with empty stomachs as an indirect measure of prey availability (as per Carline et al. 1986). We found no relationship between predatory mortality and percentage of largemouth bass with empty stomachs (range 45 – 55%) (Pearson correlation, $r = -0.49, p = 0.07$). In addition, losses from predation were not a function of largemouth bass density for either muskellunge (Pearson correlation, $r = 0.68$, $p = 0.32$) or northern pike ($r = -0.10$, $p = 0.87$), but were marginally related to largemouth bass density for tiger muskellunge ($r = 0.88$, $p = 0.05$).

Habitat selection and dispersal rates were similar among the three esocids in North Reservoir. Within 30 d following each of three stockings, >50% of all esocids (the exception being 36% of tiger muskellunge in 1983) were captured in the two vegetated transects farthest from the stocking site (Fig. 3). Because the majority of esocids occurred here, an area that occupied only 28% of the reservoir, all three esocids preferred vegetated habitats. Dispersal rates within each of the three stockings did not differ among esocids (Fig. 3, ANCOVA, homogeneity of slope, $p > 0.23$).

Pond Experiments

To compare data collected from ponds for all 3 yr, we estimated control mortality for 1983 experiments using mean mortality in controls from other years ($\bar{x}$ = 16%, 95% confidence interval = ±1.3). Because mortalities were expressed as percentages, we used arcsin transformations to stabilize the variance (Steel and Torrie 1980). Experiments were blocked (N = 4) to account for effects of esocid size and year. Differences in mortalities between treatment (largemouth bass present) and control (no largemouth bass) were used to assess losses of esocids to predation. Tiger muskellunge were more susceptible to largemouth bass predation than muskellunge (two-way ANOVA, $p = 0.04$, Tukey's multiple comparisons, $p = 0.08$); although not different from either tiger muskellunge or muskel-

TABLE 3. Number of tiger muskellunge (TM), northern pike (NP), and muskellunge (M) recovered from largemouth bass stomachs following stocking in each reservoir. Estimates of predatory mortality were obtained on each sampling date by multiplying the number of each esocid per bass stomach by the number of largemouth bass in the population (see Table 2). Interpolating between sample dates and summing these daily values provided an estimate of the total number of esocids eaten. Northern pike were not stocked in North Reservoir in 1983.

Reservoir (year)	No. of largemouth bass examined	No. of esocids recovered from bass stomachs			Estimated no. of esocids eaten		
		TM	NP	M	TM	NP	M
North (1983)	662	18	—	11	270	—	298
Kokosing (1984)	425	14	11	4	610	308	98
North (1984)	524	8	5	3	945	278	61
Madison (1984)	758	29	23	19	402	349	234
North (1985)	283	1	1	1	23	37	37

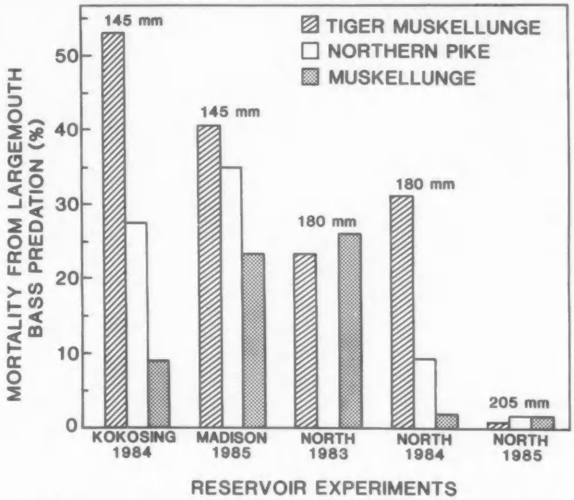

FIG. 2. Mortality for each esocid taxon attributable to largemouth bass predation following five reservoir experiments. Mean total lengths of esocids are given for each stocking. Northern pike were not stocked in North Reservoir in 1983.

lunge, northern pike were intermediate in susceptibility to predation. We found no consistent relationship between mortality and size either within or among esocid taxa. These patterns were strongly influenced by the use of largemouth bass with different levels of acclimation to ponds. Largemouth bass collected from hatchery ponds in 1985 were better acclimated to ponds than the reservoir largemouth bass used in the previous 2 yr. Before esocid introductions, 100% of largemouth bass examined contained food ($\bar{x}$ = 5.7 g food/bass) in 1985 as compared with 64% ($\bar{x}$ = 1.4 g/bass) in the other 2 yr. As a result, predation rates by these acclimated bass were higher. Comparing only experiments with similarly acclimated largemouth bass, larger esocids suffered lower predatory mortality (two-way ANOVA, $p < 0.04$, Tukey's multiple comparisons, $p < 0.05$). We also assessed losses of esocids to predation in pond experiments by directly pumping largemouth bass stomachs. In general, the number of esocids per largemouth bass stomach peaked on the first day following stocking and gradually declined over 9 d (Fig. 4). As in reservoir experiments, we used the number of esocids recovered from largemouth bass stomachs to obtain daily estimates of losses to predation for each esocid (Table 4). Again, tiger muskellunge were more susceptible to largemouth bass predation than muskellunge (ANOVA, $p = 0.01$, Tukey's multiple comparisons, $p = 0.02$); northern pike were intermediate in susceptibility to predation. Estimates of largemouth bass predation, determined by differences in mortality between treatment and control and by examination of largemouth bass stomach contents, were similar for five of the seven replicates (Table 4). Discrepancies between the two methods of estimating losses to predation occurred in the first two replicates of 1985, where substantially lower estimates of muskellunge mortality resulted from examination of largemouth bass stomachs compared with the number of fish remaining at the end of the experiment. During snorkel transects, we found four and five dead muskellunge, respectively, in the largemouth bass half of each of these two experiments. Deleting these two experiments, results were similar between

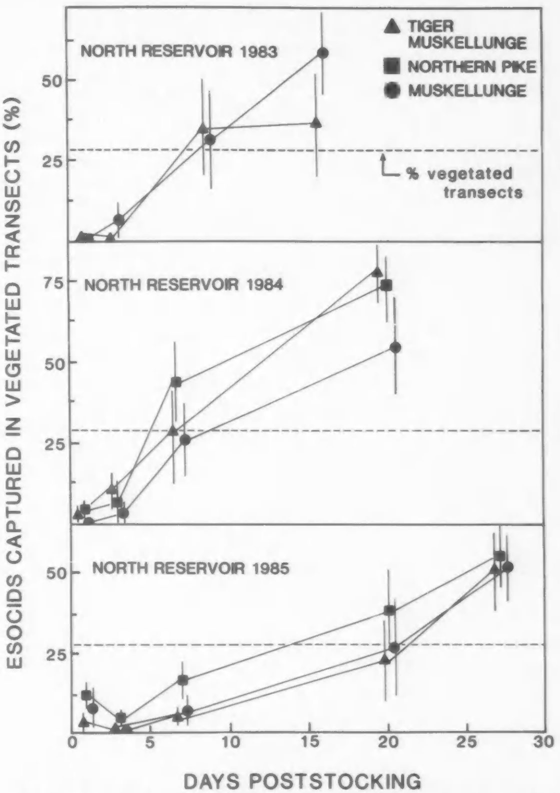

FIG. 3. Percent of each esocid taxon captured in the two vegetated transects ($N = 7$ total transects) following three stockings in North Reservoir. Vertical lines represent 95% confidence intervals. Movement rates among taxa into these two transects (located farthest from the stocking site) did not differ in any year (ANCOVA, homogeneity of slope, $p > 0.23$).

TABLE 4. Mortality among tiger muskellunge (TM), northern pike (NP), and muskellunge (M) attributable to largemouth bass predation after 9 d in 0.05-ha ponds. Mortality estimates were derived by difference in mortality between treatment (bass present) and control (no bass) pond halves and by directly pumping largemouth bass stomachs. Experiments in 1983 consisted of treatment-only ponds; control mortality was estimated using mean mortality in controls from other years. Esocid total lengths (mm) represent all fish used in experimental replicates (N = 100/taxon).

Year	Esocid total length (mm) (95% C.I.)	Treatment minus control mortality (%)			Recovered from bass stomachs (%)		
		TM	NP	M	TM	NP	M
1983	136 (±0.9)	47	29	7	43	25	0
1984	169 (±0.9)	38	18	0	23	16	0
	170 (±0.9)	22	10	4	17	4	4
1985	172 (±0.8)	54	58	78	65	34	44
	172 (±0.9)	38	48	66	26	40	19
	202 (±0.7)	38	32	12	49	48	16
	201 (±0.7)	36	30	42	46	18	39

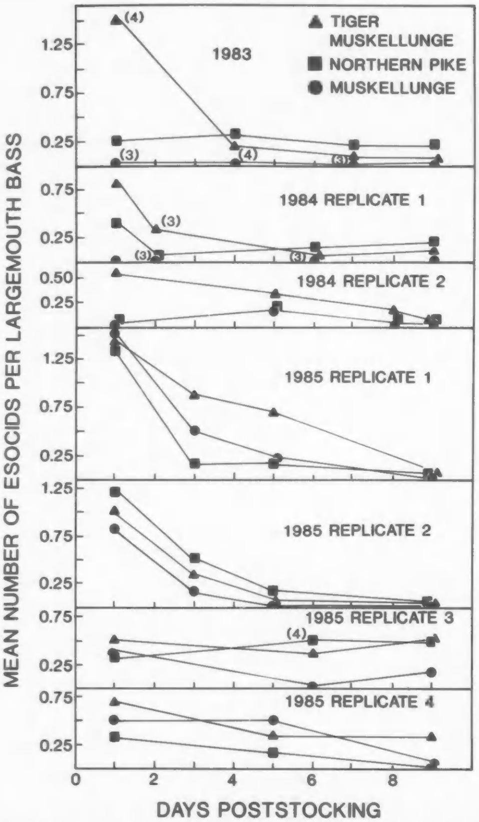

FIG. 4. Mean number of esocids recovered from largemouth bass stomachs following stocking in seven replicate pond experiments completed during 3 yr. Numbers of largemouth bass (3–10), collected by seining, are given in parentheses only when sample sizes were <5.

the two methods of estimating losses to largemouth bass predation.

We examined habitat selection by esocids by swimming transects. Without exception, esocids resided near the perimeter of the pond (about 0.5 m deep). Tiger muskellunge (N = 95 observations) spent more time in the open than either northern pike (N = 27) or muskellunge (N = 58) (Fig. 5, chi-square, partitioned degrees of freedom, $p < 0.005$). In contrast, northern pike were most often associated with the bottom of the pond whereas tiger muskellunge and muskellunge spent the majority of their time in the upper portion of the water column (Fig. 5, chi-square, $p < 0.005$).

Laboratory Experiments

In the laboratory, we assessed habitat selection and location in the water column for comparison with field results. All esocids spent the majority of their time in the vegetated portion of the pool; tiger muskellunge spent more time in the open than either muskellunge or northern pike (Fig. 5, ANOVA, Tukey's multiple comparisons, $p = 0.0001$). As in pond experiments, northern pike were more often associated with the substrate than either muskellunge or tiger muskellunge (Fig. 5, ANOVA, Tukey's multiple comparisons, $p = 0.001$).

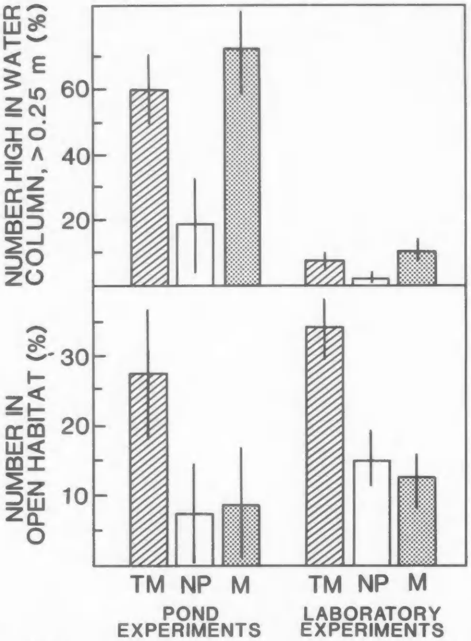

FIG. 5. Habitat selection (open or vegetated) and location in the water column (upper 0.25 m or lower 0.25 m) for each esocid taxon during pond and laboratory experiments. Values for pond experiments represent percent of total number of observations per taxa during snorkel transects. Observations for laboratory experiments (N = 106 replicates) were recorded at 2-min intervals during 1 h before largemouth bass release. Vertical lines represent 95% confidence intervals.

After largemouth bass release, we quantified esocid response to largemouth bass. Esocid movement increased following largemouth bass release (t-test, $p < 0.001$); number of position changes increased more (156% increase) for northern pike than for either muskellunge (62%) or tiger muskellunge (31%) (ANOVA, Tukey's multiple comparisons, $p < 0.05$). No difference in distances maintained from largemouth bass occurred either among taxa within a habitat (Table 5, ANOVA, $p < 0.32$) or between open and vegetated habitats (t-test, $p < 0.13$). However, flight distances were greater in open than in vegetated habitats for all esocids (t-test, $p < 0.02$) and were greater for northern pike than for the other taxa within a habitat (Table 5, ANOVA, $p < 0.001$). We also recorded the number of times each esocid failed to flee as a largemouth bass approached within 30 cm. Tiger muskellunge remained motionless more often (48%) than either muskellunge (23%) or northern pike (14%) (chi-square, partitioned degrees of freedom, $p < 0.005$).

Largemouth bass followed all three esocids more often in open than in vegetated habitats (Table 5, t-test, $p < 0.05$); however, we observed no difference among taxa within a habitat (ANOVA, $p = 0.44$). Largemouth bass followed tiger muskellunge greater distances in open than in vegetated habitats (Table 5, t-test, $p < 0.02$), but followed the other taxa equal distances (t-test, $p < 0.37$). Within a habitat, largemouth bass followed northern pike for greater distances than the other two taxa when they were in the vegetation (ANOVA, $p = 0.03$), but not in the open (ANOVA, $p = 0.15$). After 24 h, all esocids were equally susceptible to largemouth bass predation in laboratory pools (Fig. 6, chi-square, $p = 0.15$). Comparison of

TABLE 5. Comparison of esocid behaviors (recorded at 2-min intervals) in open and vegetated habitats during 1-h experiments ($N = 106$) following release of largemouth bass into pools. Mean flight distance was based on a subset of 30 experiments whereas bass behavior (distance and number of follows) represents means for individual bass ($N = 15$). Number in parentheses represent 95% confidence intervals. Row values followed by the same letter do not differ (ANOVA, Tukey's multiple comparisons, $p < 0.05$).

		Esocid taxa		
Esocid/bass behavior	No. of replicates	Tiger muskellunge	Northern pike	Muskellunge
Distance to bass (cm)	106			
Open		56.2 (±7.5) a	60.5 (±8.6) a	49.4 (±9.2) a
Vegetated		62.9 (±5.2) a	67.3 (±5.3) a	62.3 (±5.8) a
Flight distance (cm)	30			
Open		29.1 (±6.7) a	47.0 (±6.7) b	27.1 (±6.3) a
Vegetated		18.7 (±3.3) a	29.9 (±4.6) b	16.6 (±3.3) a
Mean number of follows	15			
Open		1.8 (±0.2) a	1.7 (±0.1) a	1.5 (±0.2) a
Vegetated		1.4 (±0.2) a	1.4 (±0.2) a	1.2 (±0.1) a
Distance followed (cm)	15			
Open		83.6 (±22.5) a	70.5 (±23.9) b	50.1 (±21.1) a
Vegetated		40.8 (±19.9) a	87.2 (±23.3) b	37.9 (±21.1)a

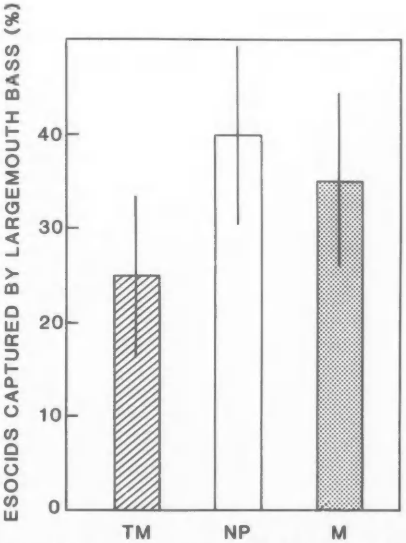

FIG. 6. Percent of each esocid taxon captured by largemouth bass after 24 h ($N = 106$ experiments) in laboratory pools (2.5 m in diameter). Vertical lines represent 95% confidence intervals. No difference in susceptibility to predation was observed among esocid taxa (chi-square, $p = 0.15$).

mean number of captures for individual largemouth bass also indicated no difference among taxa (Friedman rank sums, $p = 0.18$).

Discussion

In both pond and reservoir experiments, we found that tiger muskellunge were most susceptible to largemouth bass predation, followed by northern pike and muskellunge. Losses to predation determined by examination of largemouth bass stomachs were confirmed in pond experiments by actual esocid survival. Direct estimates of predation losses were more reliable, as other sources of mortality (handling or other predators, such as birds) were important in at least some ponds. In reservoir experiments, the magnitude of predation mortality determined for tiger muskellunge (1 – 53%) was similar to that observed in previous reservoir studies (0 – 45%) (Stein et al. 1981; Carline et al. 1986).

Size, presence of alternate prey, and density of largemouth bass may influence predation on tiger muskellunge (Stein et al. 1981; Carline et al. 1986) and muskellunge (Johnson 1982; Serns and Andrews 1986). For all taxa in reservoirs, predatory losses were least for the largest size class stocked. For smaller esocids, the number of largemouth bass in a population capable of ingesting them was higher than for larger esocids. Bass-related mortality increased as size of tiger muskellunge and northern pike decreased. However, low predatory mortality for muskellunge in one experiment with small esocids (Kokosing Reservoir) and few stockings ($N = 5$) combined to yield no significant relationship with size for this taxon. Size also was important in pond experiments; larger esocids suffered lower predatory mortality.

Availability of alternate forage and predator density did not appear to influence predation mortality in reservoirs. Losses of esocids to predators were unrelated to either percentage of largemouth bass with empty stomachs or density of largemouth bass. In contrast, Carline et al. (1986) found positive correlations for tiger muskellunge, concluding that largemouth bass were most likely to eat recently stocked hybrids when other prey were not abundant and predator densities were high. However, their conclusions were affected strongly by data from Bird's Pond in which largemouth bass were food limited (~80% empty stomachs) and dense (~75/ha). If we limit comparisons with Carline et al. (1986) to congruent ranges of predator densities and empty stomachs (1 – 12/ha and 40 – 60% empty), then predation intensity in both studies becomes unrelated to either predator density or empty stomachs.

To explain differential susceptibility among these esocids, we evaluated taxon-specific habitat selection and movement patterns following stocking. In all systems (reservoirs, hatchery ponds, and laboratory pools), esocids preferred vegetated habitats. Similarly, young-of-year northern pike in the Mississippi River prefer vegetation (Holland and Huston 1984). Tiger muskellunge spent more time in open habitats than the other two taxa. Also, tiger muskellunge and muskellunge spent more time suspended in the water column whereas northern pike were associated with the substrate. These differences in habitat selection may be caused by differences in rearing environment. Because of their experience with pellets dropped from above, tiger muskellunge may reside in open water near the surface following stocking. Differences in habitat use may affect predation risk in esocids as has been observed previously in communities of rodents and sunfishes (Werner et al. 1983; Kotler 1984; Mittelbach 1984). Habitat heterogeneity reduces predation rates by piscivores (Nyberg 1971; Mittelbach 1984; Savino and Stein 1982); prey which remain in the open, such as tiger muskellunge, are more vulnerable to predation.

We also evaluated if differential dispersal could influence susceptibility to largemouth bass. In previous work, predation by largemouth bass correlated strongly with movement of tiger muskellunge (Stein et al. 1981). As hybrids moved through the reservoir and were exposed to a progressively larger fraction of the largemouth bass population, predation mortality increased. Because dispersal rate following stocking did not differ among taxa, this is not a likely explanation for differential mortality.

In contrast with field results, laboratory experiments revealed no difference in susceptibility to predation. Behavior important in determining predation in the laboratory may not be important in the field where predators and prey are not confined by pool walls. Experimental pools were small, allowing largemouth bass to search the entire system frequently. As a result, position changes by esocids in an attempt to avoid predator encounters increased dramatically over those observed in the absence of largemouth bass. In the laboratory, largemouth bass are more likely to attack prey in closer proximity or with greater motion (Howick and O'Brien 1983). Although distances maintained by esocids from largemouth bass did not differ across taxa or habitats, flight distances did vary, being greater for all taxa in open habitats. As a result, largemouth bass followed esocids more frequently in open habitats. Whereas increased motion may attract largemouth bass in pools, it may not be important in the reservoirs and, in fact, may facilitate escape. In fact, failure to flee in the field may allow a searching predator to approach within striking distance. Tiger muskellunge failed to react to an approach by largemouth bass more frequently than either muskellunge or northern pike. Because tiger muskellunge held in hatchery troughs become acclimated to human movements, they may startle less easily than muskellunge and northern pike reared in ponds. Escape and startle responses habituate in fish (Rodgers et al. 1963) and may increase suceptibility of tiger muskellunge to predation.

Esocid behavior provides some insight into mechanisms of differential susceptibility to predation. Movement rates and distance from the predator were similar among the three taxa and flight distance did not vary predictably with vulnerability to predation. In contrast, habitat selection and flight response differed between tiger muskellunge and its parents. Tiger muskellunge spent more time in open habitats and failed to flee more frequently than muskellunge or northern pike. These behavioral differences explain, at least partially, the increased vulnerability of tiger muskellunge to predation.

Management Recommendations

Behavioral differences among these esocids may be innate or related to differences in rearing environment. Recent efforts to rear northern pike and muskellunge on pelleted diets in hatchery troughs have become increasingly successful (S. Hood, PA Fish. Comm., pers. comm.). Forney and McKeown (1988) documented lower survival for pellet-reared muskellunge than for pond-reared fish, supporting the hypothesis that rearing environment can contribute to success. However, intensively reared muskellunge were stocked earlier in the year and at smaller sizes than pond-reared fish, factors we found critical to survival. Mechanisms of mortality in intensively versus extensively reared esocids must be understood before the contribution of rearing environment to survival can be assessed.

Many factors influence where esocids should be stocked, including presence of appropriate forage (Carline et al. 1986; Wahl and Stein 1988), availability of suitable habitat (Dombeck et al. 1986), as well as density and size structure of resident predators. Managers must decide the time of year, size, and taxon of esocid to stock. We reduced losses to largemouth bass predation by stocking all three esocids in fall when water temperatures were <25°C and when esocid sizes were >205 mm. From their work with tiger muskellunge, Stein et al. (1981) concluded that 250-mm fish would be relatively free from predation. Tiger muskellunge were more susceptible to predation than northern pike or muskellunge in our experiments, suggesting that stocking sizes for the hybrid should be larger than for the other two species when predators are abundant. Fall stockings will increase survival of esocids for several reasons including dispersal of predators following destratification (Stein et al. 1981), reduced food consumption by largemouth bass at temperatures <25°C (Rice and Cochran 1984), and reduced thermal stress caused by differences in temperatures between hatchery and lake (Mather and Wahl 1989).

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References

BEAMISH, F. W. H. 1972. Ration size and digestion in largemouth bass, *Micropterus salmoides* Lacepede. Can. J. Zool. 50: 153–164.

BELUSZ, L. C. 1978. An evaluation of the muskellunge fishery of Lake Pomme de Terre and efforts to improve stocking success. Am. Fish. Soc. Spec. Publ. 11: 292–297.

CARLINE, R. F., R. A. STEIN, AND L. M. RILEY. 1986. Effects of size at stocking, season, largemouth bass predation, and forage abundance on survival of tiger muskellunge. Am. Fish. Soc. Spec. Publ. 15: 151–167.

Crossman, E. J., and J. M. Casselman. 1969. Identification of northern pike and muskellunge from axial skeletons, scales, and epipleurals. J. Fish. Res. Board Can. 26: 175–178.

Crowder, L. B., and W. E. Cooper. 1982. Habitat structural complexity and the interaction between bluegills and their prey. Ecology 63: 1802–1813.

Dombeck, M. P., B. W. Menzel, and P. N. Hinz. 1986. Natural muskellunge reproduction in midwestern lakes. Am. Fish. Soc. Spec. Publ. 15: 122–134.

Forney, J. L., and P. E. McKeown. 1988. Status of fish stocks in Chautauqua Lake 1987. New York Department of Environmental Conservation, Federal Aid in Fish Restoration Project 7, Albany, NY.

Foster, J. R. 1977. Pulsed gastric lavage: an efficient method of removing the stomach contents of live fish. Prog. Fish-Cult. 39: 166–169.

Graff, D. R. 1978. Intensive culture of esocids: the current state of the art. Am. Fish. Soc. Spec. Publ. 11: 195–201.

Headrick, M. R. 1985. Bioenergetic constraints on habitat use by northern pike (*Esox lucius*) in Ohio reservoirs. Doctoral dissertation, The Ohio State University, Columbus, OH.

Holland, L. E., and M. L. Huston. 1984. Relationship of young-of-the-year northern pike to aquatic vegetation types in backwaters of the Upper Mississippi River. N. Am. J. Fish. Manage. 4: 514–522.

Howick, G. L., and W. J. O'Brien. 1983. Piscivorous feeding behavior of largemouth bass: an experimental analysis. Trans. Am. Fish. Soc. 112: 508–516.

Hunt, B. P. 1960. Digestion rate and food consumption of Florida gar, warmouth and largemouth bass. Trans. Am. Fish. Soc. 89: 206–211.

Hurlbert, S. H., J. Zedler, and D. Fairbanks. 1972. Ecosystem alteration by mosquito fish (*Gambusia affinis*) predation. Science (Wash., DC) 175: 639–641.

Johnson, L. D. 1978. Evaluation of esocid stocking program in Wisconsin. Am. Fish. Soc. Spec. Publ. 11: 298–301.

1982. Factors affecting short-term survival of stocked muskellunge fingerlings in Wisconsin. Wisconsin Department of Natural Resources, Federal Aid in Fish Restoration Project F-83, Madison, WI.

Kotler, B. P. 1984. Risk of predation and the structure of desert rodent communities. Ecology 65: 689–701.

Macan, T. T. 1977. The influence of predation on the composition of freshwater animal communities. Biol. Rev. 52: 45–70.

Mather, M. E., and D. H. Wahl. 1989. Comparative mortality in three esocids due to stocking stress. Can. J. Fish. Aquat. Sci. 46: 214–217.

Mittelbach, G. G. 1984. Predation and resource partitioning in two sunfishes (Centrarchidae). Ecology 65: 499–513.

Morin, P. J. 1984. The impact of fish exclusion on the abundance and species composition of larval odonates: the results of short-term experiments in a North Carolina farm pond. Ecology 65: 53–60.

Nyberg, D. W. 1971. Prey capture in the largemouth bass. Am. Midl. Nat. 86: 128–144.

Peckarsky, B. L., and S. I. Dodson. 1980. Do stonefly predators influence benthic distributions in streams? Ecology 61: 1275–1282.

Rice, J. A., and P. A. Cochran. 1984. Independent evaluation of a bioenergetics model for largemouth bass. Ecology 65: 732–739.

Ricker, W. E. 1985. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191.

Rodgers, W. L., R. Melzack, and J. R. Segal. 1963. 'Tail flip response' in goldfish. J. Comp. Physiol. Psychol. 56: 917–923.

Savino, J. F., and R. A. Stein. 1982. Predator–prey interaction between largemouth bass and bluegills as influenced by simulated, submerged vegetation. Trans. Am. Fish. Soc. 111: 255–266.

Serns, S. L., and L. M. Andrews. 1986. Comparative survival and growth of three sizes of muskellunge fingerlings stocked in four northern Wisconsin lakes. Am. Fish. Soc. Spec. Publ. 15: 229–237.

Steel, R. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. McGraw Hill, New York, NY.

Stein, R. A., R. F. Carline, and R. S. Hayward. 1981. Largemouth bass predation on stocked tiger muskellunge. Trans. Am. Fish. Soc. 110: 604–612.

Wahl, D. H., and R. A. Stein. 1987. Application of liquid oxytetracycline in formulated feeds to mark and treat tiger muskellunge (northern pike × muskellunge). Prog. Fish-Cult. 49: 312–314.

1988. Prey selection by three esocids: the role of prey behavior and morphology. Trans. Am. Fish. Soc. 117: 142–151.

Weithman, A. S., and R. O. Anderson. 1977. Survival, growth, and prey of Esocidae in experimental systems. Trans. Am. Fish. Soc. 106: 424–430.

Werner, E. E., J. F. Gilliam, D. J. Hall, and G. G. Mittelbach. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology 64: 1540–1548.

Freezing Resistance in Some Northern Populations of Pacific Herring, *Clupea harengus pallasi*

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Raymond, J. A. 1989. Freezing resistance in some northern populations of Pacific herring, *Clupea harengus pallasi*. Can. J. Fish. Aquat. Sci. 46: 2104–2107.

Pacific herring, *Clupea harengus pallasi*, were collected at three locations in Alaska and Japan in winter and spring to determine their degree of freezing resistance. Herring collected from waters whose temperatures ranged between 4.7 and −1.4°C showed serum freezing points between −1.22 and −1.40°C. All freezing points were below those expected from measurements of serum osmolality, indicating that a noncolligative antifreeze was present that added between 0.28 and 0.61° to the freezing point depression. In addition, osmotically active substances other than sodium chloride contributed to the freezing point depression in some of the samples.

Le hareng du Pacifique, *Clupea harengus pallasi*, a été échantillonné à trois stations en Alaska et au Japon durant l'hiver et le printemps afin de déterminer sa résistence au gel. Le hareng prélevé dans des eaux variant entre 4,7 et −1,4°C avait un point de congélation du sérum qui variait entre −1,22 et −1,40°C. Tous les points de congélation étaient inférieurs à ceux auxquels on pouvait s'attendre par des mesures de l'osmolalité du sérum; cela indique la présence d'un antigel non coagulant qui provoquait un abaissement supplémentaire du point de congélation de 0,28 et 0,61°. En outre, les substances actives sur le plan osmotique autres que le chlorure de sodium contribuaient à l'abaissement du point de congélation chez certains échantillons.

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Seasonal movements of Pacific herring, *Clupea harengus pallasi*, are influenced by several factors, such as currents, temperature and food availability. Among northern populations, temperature appears to play an important role. In the Bering Sea, for example, the major herring population moves off the eastern shelf as shelf waters begin to freeze, and returns soon after the spawning grounds become ice free (Wespestad and Barton 1981). During cold winters, the wintering grounds are displaced to the south (Shaboneev 1965) and the timing of the onshore spawning migration is delayed (Prokhorov 1968; Wespestad and Barton 1981). The wintering grounds of Atlantic herring, *Clupea harengus harengus*, in the Norwegian Sea appear to be similarly influenced by temperature (L. R. Shmarina, cited in Parrish and Saville 1965). Despite this apparent aversion to cold water, herring populations are found throughout the Arctic (Hay 1985), and several workers have reported Pacific herring in waters that are ice covered or at subzero temperatures (Rumyantsev and Darda 1970; Wespestad and Barton 1981; Whitmore and Bergstrom 1983). Some of the northern populations appear to be nonmigratory and spend the winter locally in or near brackish lagoons and bays.

In northern marine waters, temperatures can fall below the normal serum freezing point of teleost fishes. Some fishes avoid freezing by living in deep, ice-free waters in a supercooled state (Scholander et al. 1957), while others have developed means for lowering serum freezing points. One such means relies on an increase in osmolality of a fish's body fluids through increases in electrolytes and other solutes (Pearcy 1961; Smith and Paulson 1977; O'Grady and DeVries 1982). This type of freezing point depression is a colligative effect in that it is a function of only the number of molecules in solution. Another means of lowering freezing points relies on the production of macromolecular "antifreezes" (DeVries 1983). The antifreezes act in a noncolligative way by absorbing to the ice surface and preventing it from growing (Raymond and DeVries, 1977). A peculiarity of the antifreezes is that they lower the temperature at which a seed crystal of ice will grow but they do not lower the melting point. Thus, their presence may be detected by a thermal hysteresis, i.e. a difference between the freezing point and melting point. Eastman et al. (1987) reported a "small amount" of hysteresis in Pacific herring collected in the Bering Sea in spring. Another clupeid, *Alosa pseudoharengus*, collected in Nova Scotia was also found to have a hysteresis (Duman and DeVries 1975).

These and previous observations of Pacific herring in potentially freezing waters raise two questions. First, to what extent do herring seek refuge in brackish waters that are hypoosmotic to their own body fluids, and thus avoid a need for other types of freezing resistance? Second, if the herring do inhabit potentially freezing waters, what are the relative importance of colligative and noncolligative freezing resistance mechanisms? In the present study, herring were collected from three locations in Alaska and Japan in winter and spring to determine their environment and to determine what types of freezing resistance, if any, might be employed.

Materials and Methods

At Kotzebue, Alaska (66°51′N, 162°40′W), herring were captured with a gill net set vertically through a single hole in the ice approximately 100 m offshore. The effective width of the net was probably only about half its full width of 2 m, since the net was not extended with a frame. The net was pulled approximately every 12 h and the fish were alive and in good condition. Water temperature and salinity were measured with a YSI salinometer. At Unalakleet, Alaska (63°52′N, 160°50′E), herring were captured with a floating gill net and removed within 30 min. Near Kushiro, Japan (42°41′N, 144°45′W), herring were caught with a bottom trawl. Temperature was measured with an expendable bathythermograph (XBT).

At each location, fish were placed in a water-filled cooler until ready for bleeding. The fish were bled usually within an hour of capture. Blood was taken from the bulbus arteriosus with a 23-guage needle and 1-ml syringe. One catch at Unalakleet was too large for individual bleeding, however. These fish, about half of which had been dead for about 30 min, were bled by cutting off the tail and allowing the blood to drip into a bucket. The blood samples were pooled, allowed to clot for approximately 4 h at 0°C , and then centrifuged for approximately 10 min at 2000 rpm. Serum was removed and frozen at −20°C until ready for analyses. The Kotzebue blood samples were put in two pools (I and II) depending on whether the blood was taken indoors or on the ice, respectively. The Unalakleet samples were also placed in two pools depending on whether the blood was needle bled (I) or drip bled (II).

Freezing points were measured with the capillary freezing point method (DeVries 1983). The bath temperature was typically lowered about 0.05° every 5 min and the seed crystal was observed for growth. The freezing point apparatus is capable of detecting growth of 50 μm or more, so that at the above cooling rate, growth rates less than about 2×10^{-5} cm/s would not have been detected. Occasionally the temperature was held constant for longer periods, enabling slower growth rates to be detected. If growth was observed, the temperature would be held constant. The "freezing points," which were not true equilibrium freezing points, were defined as the highest temperature at which continued growth was observed. The freezing points reported here represent the average of at least two determinations.

Osmolality was measured with a Wescor model 1500c vapor pressure osmometer. Melting points were calculated from the osmometry readings using the conversion −1.858° melting point depression per 1000 mosmol/L. Chloride was measured with a Buchler chloridometer. Sodium and potassium were measured with a Corning model 455 flame photometer. Serum was dialized against deionized water at 4°C using Spectrapor 6 tubing.

Results

Environments

At Kotzebue, 33 herring were captured from under the ice between 26 April and 1 May near the mouth of a brackish water inlet. The fish were caught throughout the water column (0–23 m). Water temperatures and salinities ranged from approximately −1.0°C and 10 ppt at the surface to −1.5°C and 25 ppt at 14 m, the maximum depth that the salinometer sensor could reach. Water temperatures measured at the depth and time of capture ranged from −1.1 to −1.5°C.

At Unalakleet, water temperature was 4.7°C at the surface and 4.4°C at a depth of 8 m on 9 June, the day herring were collected. This was about 2 wk after the breakup of the ice cover; large ice packs were still common in the area at the time of capture.

At Kushiro, herring were caught on or near the bottom at a depth of 166 m on 21 February. An XBT deployed nearby in water whose depth was 176 m indicated water temperatures of −1.1°C at the surface, increasing linearly to +0.5°C at 166 m and +1.3°C at the bottom.

Each population appeared to have access to waters whose temperatures were both above and below the temperature at which most teleost fish would be expected to freeze. The habitat of the Kotzebue herring indicated that some populations were able to enter potentially freezing waters, at least for short periods.

Blood Analyses

Melting points of the different serum samples varied from −0.61°C to −1.02°C, with the lower values being found in the Japanese fish (Table 1). Most of the melting point depression in the Alaskan fishes could be attributed to electrolytes. In the Japanese herring, however, other substances appeared to be present, since the Na, Cl, and K accounted for only about three quarters of the melting point depression. The melting points may have been slightly lowered by the stress caused by capture and holding of the fish (Umminger 1970).

Seed crystals in each sample of herring serum did not begin to show observable growth until the temperature was lowered significantly below the melting point. The exact freezing points were not well-defined, but fell consistently within a range of ±0.1° of that shown in Table 1. Often the seed would grow a minute amount following each temperature decrease and then appear to stop growing. Occasionally, very slow growth occurred when the temperature was held constant for longer periods, making it difficult to distinguish between a true freezing point depression and a pronounced decrease in the growth rate. For example, an ice seed immersed in Unalakleet II serum at −1.20°C grew about 1.5 mm over a 48-h period. In another instance, an ice seed in Kushiro herring serum at −1.38°C grew a 1-mm-long hexagonal prism in the first 3 h, but no further growth was observed in the following 20 h.

The difference between melting point and freezing point of the samples of herring serum indicates the presence of a noncolligative antifreeze that provides between 0.28 and 0.61° of additional freezing protection. A dialized sample of Unalakleet II serum showed a melting point–freezing point hysteresis of about 0.10° while the dialysate showed a hysteresis of less than 0.02° (values stated to correct for dilutions). This suggests that the antifreeze has a molecular mass greater than the dialysis tubing cutoff, which is about 1000 Da. The reason for the reduction in hysteresis after dialysis is not clear, but it may have been due to partial precipitation of the antifreeze. Another indication that the antifreeze is a macromolecule was its degree of precipitation under ultracentrifugation: after centrifugation of a Unalakleet serum sample for 17 h at 80 000 rpm, serum from the top, middle, and bottom thirds of the tube showed thermal hystereses of 0.08, 0.14, and 0.33°, respectively. After a roughly similar centrifugation (13.5 h at 60 000 rpm), the serum of another antifreeze-bearing fish, the antarctic *Dissostichus mawsoni*, showed a roughly similar distribution of hysteresis. The latter antifreeze consisted of several fractions ranging in molecular mass from 2400 to 34 000 Da (DeVries 1983).

Ice crystals grown in the presence of herring serum did not produce the long *c*-axis needles or pits on the basal plane that

TABLE 1. Serum parameters of Pacific herring from Alaska and Japan. VP is the osmolality (mosmol/L) measured with a vapor pressure osmometer. MP and FP are the melting and freezing points.

Location		Water temp. (°C)	No. in pool	Na	Cl	K	VP	MP[a]	MP	FP	Hysteresis[b]
					(mequiv.L)						
Kotzebue	I	−1.1 to −1.5	5	186	132	4.4	351	−0.65	−0.61	−1.22	0.61
	II	−1.1 to −1.5	10	208	156	3.6	440	−0.82	−0.80	−1.28	0.48
Unalakleet	I	4.4 to 4.7	8				448	−0.83		−1.40	0.57
	II	4.4 to 4.7	250	192	193	4.4	438	−0.81		−1.25	0.44
Kushiro		0.5 to 1.3	6	187	170	4.4	508	−0.94	−1.02	−1.30	0.28

[a] Calculated from the vapor pressure osmometry value based on the fact that a 1000 mosmol/L solution will have a freezing point depression of −1.858°.

[b] Based on observed melting point or, when not available, the calculated melting point.

are characteristic of other antifreezes (Raymond et al. 1989). Instead, the ice usually grew in the form of hexagonal prisms. Since ice does not normally grow in this habit from the melt, it is likely that some component in the serum is adsorbing to the ice. However, the hexagonal prism habit was shown by the serum dialysate but not the dialized serum, so it does not appear to be caused by the antifreeze retained by the dialysis tubing.

Discussion

Among polar and subpolar fishes, a variety of macromolecular antifreezes have been found; they include glycopeptides in the nototheniids and the gadids and several different peptides in the pleuronectids, cottids, and zoarcids (DeVries 1983; Schrag et al. 1987; Davies et al. 1988). The present results show that the Pacific herring also has a noncolligative antifreeze. This is demonstrated by a difference in serum melting points and freezing points of up to 0.61°. That an antifreeze is present is also suggested by the habitat of the Kotzebue herring: shallow, ice-covered waters whose temperatures and likely presence of ice particles would be lethal to most temperate water fishes.

At present, little is known about the nature of the herring antifreeze. It appears to have a molecular mass greater than 1000 Da. It differs from the other antifreezes, however, in its effects on ice. The peptide and glycopeptide antifreezes cause ice to grow in the form of elongated needles (Raymond and DeVries 1977; Schrag et al. 1987), which is an indication of a common adsorption mechanism. The herring antifreeze does not cause ice needles to form. Also, because the freezing points of the herring serum samples were not well-defined, it is unclear whether the herring antifreeze completely stopped growth, as do other antifreezes, or acted as a powerful retardant of growth.

However, this distinction may not be so important to the herring. On the occasions when slow freezing of ice crystals occurred, the temperatures corresponded to supercoolings of about 0.4°C. At this amount of supercooling, ice would normally grow at a rate of 3.4×10^{-2} cm/s (Fletcher 1970, table 5–1). However, the observed growth rates were at least four orders of magnitude slower than this. If slow growth were to occur at temperatures in the range of −1.4°C, then there would be ample time for a herring to move into warmer waters where any incipient ice particles would melt. Each of the herring samples collected in this study had such warmer waters available to them.

The melting point of the Kushiro serum sample, −1.02, was quite low compared with those of other marine teleosts (Holmes and Donaldson 1969). Increased levels of serum osmolytes in these fish, which were largely due to solutes other than NaCl, thus provided a few tenths of a degree of freezing protection in addition to that provided by the noncolligative antifreeze. A similar low melting point was obtained by Holliday and Blaxter (1963) with Atlantic herring. Trimethylamine oxide was found to increase in Atlantic herring muscle tissue in winter (Hughes 1959), but it is not known whether a corresponding increase occurred in the blood.

The lowest freezing points observed in the herring, about −1.4°C, were not low enough to protect against full-strength seawater when it is cooled to its freezing point (−1.9°C). However, occurrences of Pacific herring in waters at temperatures lower than −1.4°C have been reported. Rumyantsev and Darda (1970) found herring in the eastern Bering Sea at temperatures as low as −1.8°C. Herring had also been recovered from the stomachs of seals captured in the coastal waters of Kotzebue Sound and the southeastern Chukchi Sea in January and February (L. Lowry, Alaska Department of Fish and Game, unpubl. data). The water temperatures of the locations where these herring were caught by the seals were probably no higher than −1.7°C.

These fish may have had higher antifreeze levels than those used in the present study. They may also have been supercooled by a few tenths of a degree, although all were caught in relatively shallow waters where avoiding ice particles would be difficult. Supercooling could be aided if the herring's skin was resistant to the propagation of ice. Recently, Fletcher et al. (1988) found that some salmonids may gain as much as 0.2° of additional freezing protection by this method. It is noteworthy in this regard that herring collected in the waters of British Columbia showed a threefold increase in collagen content in the skin and scales in winter (McBride et al. 1960).

Available low temperature tolerance data on the closely related Atlantic herring are consistent with those reported here. Herring from the Barents Sea died at temperatures of −1.5°C (Kamshylov and Gerasimov 1960, cited in Holliday and Blaxter 1963). Brawn (1960) found that New Brunswick herring survived at −1.4°C for several hours, after which they were released. The lower lethal temperature of herring larvae acclimatized to 7°C was −1.8°C (Blaxter 1960).

Further studies to determine the composition and structure of the herring antifreeze are planned. In addition, other studies to determine the effects of season and latitude on antifreeze production should be useful in understanding the distribution and migratory behavior of the herring.

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References

BLAXTER, J. H. S. 1960. The effect of extremes of temperature on herring larvae. J. Mar. Biol. Assoc. U.K. 39: 605–608.

BRAWN, V. M. 1960. Temperature tolerance of unacclimatized herring (*Clupea harengus*). J. Fish. Res. Board Can. 17: 721–723.

DAVIES, P. L., C. L. HEW, AND G. L. FLETCHER. 1988. Fish antifreeze proteins: physiological and evolutionary biology. Can. J. Zool. 66: 2611–2617.

DEVRIES, A. L. 1983. Antifreeze peptides and glycopeptides in cold-water fishes. Annu. Rev. Physiol. 45: 245–260.

DUMAN, J. G., AND A. L. DEVRIES. 1975. The role of macromolecular antifreezes in cold water fishes. Comp. Biochem. Physiol. 52A: 193–199.

EASTMAN, J. T., R. B. BOYD, AND A. L. DEVRIES. 1987. Renal corpuscle development in boreal fishes with and without antifreezes. Fish Physiol. Biochem. 4: 89–100.

FLETCHER, G. L., M. H. KAO, AND J. B. DEMPSON. 1988. Lethal freezing temperatures of arctic char and other salmonids in the presence of ice. Aquaculture 71: 369–378.

FLETCHER, N. H. 1970. The chemical physics of ice. Cambridge University Press, London, 271 p.

HAY, D. E. 1985. Reproductive biology of Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 42: 111–126.

HOLLIDAY, F. G. T., AND J. H. S. BLAXTER. 1963. The behaviour and physiology of herring and other clupeids, p. 262–393. *In* F. S. Russell [ed.] Advances in marine biology. Vol. 1. Academic Press, London and New York.

HOLMES, W. N., AND E. M. DONALDSON. 1969. The body compartments and the distribution of electrolytes, p. 1–89. *In* W. S.Hoar and D. J. Randall [ed.] physiology. Vol. 1. Academic Press, New York, NY.

HUGHES, R. B. 1959. Chemical studies of the herring (*Clupea harengus*). 1. Trimethylamine oxide and volatile amines in fresh, spoiling and cooked herring flesh. J. Sci. Food Agric. 10: 431–436.

KAMSHYLOV, M. M., AND V. V. GERASIMOV. 1960. An attempt at keeping young Murman herring in aquarium, p. 84–87. Proc. Symp. on the biological bases of the oceanic fisheries. (In Russian).

MCBRIDE, J. R., R. A. MACLEOD, AND D. R. IDLER. 1960. Seasonal variation in the collagen content of Pacific herring tissues. J. Fish. Res. Board Can. 17: 913–918.

O'GRADY, S. M., AND A. L. DEVRIES. 1982. Osmotic and ionic regulation in polar fishes. J. Exp. Mar. Biol. Ecol. 57: 219–228.

PARRISH, B. B., AND A. SAVILLE. 1965. The biology of the northeast Atlantic herring populations. Oceanogr. Mar. Biol. Annu. Rev. 3: 323–373.

PEARCY, W. G. 1961. Seasonal changes in osmotic pressure of flounder sera. Science (Wash., DC) 134: 193–194.

PROKHOROV, V. G. 1968. Winter period of life of herring in the Bering Sea. Proc. Pac. Sci. Res. Inst. Fish. Oceanogr. 64: 329–338. (Transl. 1970, Fish. Res. Board Can. Transl. Ser. 1433).

RAYMOND, J. A., AND A. L. DEVRIES. 1977. Adsorption inhibition as a mechanism of freezing resistance in polar fishes. Proc. Natl. Acad. Sci. USA 74: 2589–2593.

RAYMOND, J. A., P. WILSON, AND A. L. DEVRIES. 1989. Inhibition of growth of nonbasal planes in ice by fish antifreezes. Proc. Natl. Acad. Sci. USA 86: 881–885.

RUMYANTSEV, A. I., AND M. A. DARDA. 1970. Summer herring in the eastern Bering Sea, p. 409–441. *In* P. A. Moiseev [ed.] Soviet fisheries investigations in the northeastern Pacific. Part V. (Transl. from Russian by Israel Prog. for Sci. Transl. for the National Marine Fisheries Service, 1972.)

SCHOLANDER, P. F., L. VAN DAM, J. W. KANWISHER, H. T. HAMMEL, AND M. S. GORDON. 1957. Supercooling and osmoregulation in arctic fish. J. Cell. Comp. Physiol. 49: 5–24.

SCHRAG, J. D., C. H. C. CHENG, M. PANICO, H. R. MORRIS, AND A. L. DEVRIES. 1987. Primary and secondary structure of antifreeze polypeptides from arctic and antarctic zoarcid fishes. Biochim. Biophys. Acta 915: 357–370.

SHABONEEV, I. E. 1965. Biology and fishing of herring in the eastern part of the Bering Sea, p. 130–154. *In* P. A. Moiseev [ed.] Soviet fisheries investigations in the northeastern Pacific. Part IV. (Transl. 1968, Israel Prog. Sci. Transl.)

SMITH, R. L., AND A. C. PAULSON. 1977. Osmoregulatory seasonality and freezing avoidance in some fishes from a subarctic eelgrass community. Copeia 1977 (2): 362–369.

UMMINGER, B. L. 1970. Effects of subzero temperatures and trawling stress on serum osmolality in the winter flounder *Pseudopleuronectes americanus*. Biol. Bull. 139: 574–579.

WESPESTAD, V. G., AND L. H. BARTON. 1981. Distribution, migration and status of Pacific herring, p. 509–525. *In* D. W. Hood and J. A. Calder [ed.] The eastern Bering Sea shelf: oceanography and resources. Vol. 1. Natl. Oceanic Atmos. Admin. Distributed by University of Washington Press, Seattle, WA.

WHITMORE, D. C., AND D. J. BERGSTROM. 1983. Assessment of herring (*Clupea harengus pallasi*) stocks in southeastern Chukchi Sea, 1980–1981. Informational Leaflet No. 210: 27 p. (Available from Alaska Dep. of Fish and Game, Juneau, AK)

Identification of Sockeye Salmon (*Oncorhynchus nerka*) Stocks in Mixed-stock Fisheries in British Columbia and Southeast Alaska using Biological Markers

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We demonstrate the present analytical capability and the potential for coast-wide stock identification of sockeye salmon (*Oncorhynchus nerka*) using reference sampling data from 51 principal stocks in British Columbia and Southeast Alaska situated between the Fraser and Taku Rivers. We evaluate the relative accuracy and precision of stock composition estimates from maximum likelihood mixture analysis with four types of biological markers, either alone or in combination; these include freshwater age, six scale pattern variables, the prevalence of the brain parasite *Myxobolus neurobius*, and five biochemical genetic (electrophoretic) traits. Using all markers in combination, estimates of mixing proportions for all test mixtures are acceptable for most purposes (roughly ±10% with 95% confidence) providing all samples are representative and mixture samples are large (≥300 fish). The reliability of these estimates is greatly reduced when reference samples are corrupted to simulate observed annual variation in scale pattern markers. Annual variation may preclude the use of scale pattern markers for complicated stock identification problems where representative reference samples cannot be obtained annually, or until after the fishing period. In contrast, no significant annual variation is detectable for biochemical genetic and brain parasite markers in stocks that have been sampled repeatedly. Using only these stable markers, contributions from about 15 different groups of the 51 stocks can be estimated with acceptable precision, but in general, estimates for individual stocks are unreliable.

Nous présentons la capacité analytique et le potentiel actuels d'identification des stocks de saumon nerka (*Oncorhynchus nerka*) sur l'ensemble de la côte au moyen des données de référence sur 51 stocks principaux de la Colombie-Britannique et du sud-est de l'Alaska, entre le Fraser et la rivière Taku. Nous estimons l'exactitude et la précision relatives des évaluations de la composition des stocks obtenues à partir d'une analyse des mélanges selon la probabilité maximale, analyse qui repose sur quatre types de marqueurs biologiques pris seuls ou en combinaison; ce sont l'âge en eau douce, six variables du motif des écailles, la prévalence du parasite du cerveau *Myxobolus neurobius* ainsi que cinq traits génétiques biochimiques (électrophorèse). Lorsque tous les marqueurs sont utilisés en combinaison, les évaluations des proportions de tous les mélanges d'essai sont acceptables pour la plupart des usages (±10% environ avec un niveau de confiance de 95%) pourvu que tous les échantillons soient représentatifs et que les échantillons de mélange soient importants (300 poissons). La fiabilité de ces évaluations se trouve considérablement amoindrie lorsque des échantillons de référence sont faussés afin de simuler les variations annuelles observées dans le motif des écailles. Les variations annuelles peuvent rendre impossible l'application du motif des écailles à des problèmes compliqués d'identification des stocks où des échantillons de référence représentatifs ne peuvent être constitués chaque année ou pas avant la fin de la saison de pêche. À l'inverse, on ne détecte pas de variation annuelle importante quant aux paramètres que sont les marqueurs génétiques biochimiques et les parasites du cerveau, dans les stocks qui ont été échantillonnés à plusieurs reprises. Lorsqu'on n'utilise que ces marqueurs stables, on peut identifier la contribution d'une quinzaine de groupes différents qui constituent les 51 stocks qu'on peut évaluer avec une précision acceptable, mais en général, les évaluations qui s'appliquent aux stocks individuels ne sont pas fiables.

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Fishery managers require efficient techniques for stock identification to assess the productivity or regulate harvest rates on individual fish stocks exploited together in "mixed-stock" fisheries. Biological attributes ("markers") that differ among stocks can often be used to identify individual stocks without resorting to expensive and laborious tagging studies. For example, many salmonid stocks can be distinguished because they have unique scale structure (e.g. Henry 1961; Anas and Murai 1969; Messinger and Bilton 1974; Bilton and Messinger 1975; Cook and Lord 1978; Lear and Sandeman 1980; Marshall et al. 1982, 1984, 1987; Reddin and Misra 1985). Other biological markers have also been used to identify salmonid stocks; these include morphometric characters (Fukuhara et al. 1962; Amos et al. 1963; Pearson 1964; Winans 1984; Craig 1985), meristic characters (MacCrimmon and Claytor 1985, 1986; Blouw et al. 1988), the prevalence of indicator parasites (Margolis 1963; Pippy 1980; Steer et al. 1986; Bailey and Margolis 1987; Moles et al. 1989) and biochemical genetic differences (Child 1980; Payne 1980; Beacham et al. 1985a,b, 1987; Okazaki 1985; Wood et al. 1987b).

Fisheries agencies in Canada and the United States are engaged in cooperative research on stock identification of sockeye salmon (*Oncorhynchus nerka*) under the auspices of the Pacific Salmon Commission. To date this research has focussed on estimating stock composition in mixed-stock fisheries in northern British Columbia and Southeast Alaska using four types of biological markers — scale patterns, age composition, parasite prevalence, and biochemical genetic (electrophoretic) data (Wood et al. 1988). These "coast-wide" mixed-stock fisheries present special problems for stock composition analysis because a large number of different stocks are exploited and the proportion of the catch contributed by each stock usually varies seasonally owing to the migratory habits of sockeye salmon.

Typically, individual biological markers show only limited variation among neighbouring populations and this tends to restrict the number of stocks that can be distinguished reliably. The number of stocks that can be identified cannot exceed the number of observable states for the marker(s) being used (Pella and Milner 1987). Thus, analysts have often been forced to simplify stock identification problems by assuming a priori which stocks are likely to contribute to a mixed-stock fishery, or by aggregating individual stocks into stock-groupings until the number of groups to be identified no longer exceeds the number of possible states of the marker being used (e.g. Marshall et al. 1984). However, recent advances in computational capabilities and the application of maximum likelihood statistical models (Milner et al. 1981, 1983; Fournier et al. 1984; Pella and Milner 1987; Millar 1987) have made it feasible to use a variety of different markers in combination (Wood et al. 1987a,b). This procedure greatly increases the number of stocks that can be considered during analysis and reduces the risk of error associated with ignoring "unimportant" stocks.

In this paper, we demonstrate the present capability and the potential for coast-wide stock identification of sockeye salmon stocks using maximum likelihood mixture analysis techniques and "reference" sampling data from 51 principal sockeye stocks in British Columbia and Southeast Alaska situated between the Fraser and Taku Rivers. Monte Carlo simulations were conducted to evaluate the relative accuracy and precision of stock composition estimates for mixtures of known composition using a variety of biological markers, either alone or in combination; these markers included freshwater age, six scale pattern variables, the prevalence of the brain parasite *Myxobolus neurobius*, and five biochemical genetic traits.

We assess the potential of current methods under three scenarios. In the first scenario, we simulated ideal conditions reasoning that if the resulting estimates were unreliable, practical application would be futile. Accordingly, we ensured that (1) all reference samples were random and representative of the stocks, (2) genotype frequencies were in Hardy-Weinberg equilibrium, (3) scale pattern variables were normally distributed, and (4) mixture samples were relatively large ($n = 300$, all from a single stock).

In the second scenario, we examined the effect of using reference samples in which scale pattern data have been "corrupted" to simulate the effect of annual variation in scale patterns. These effects are of practical importance when stock composition is estimated using reference samples collected in previous years, as is necessary for most analysis during the fishing season. We also show that in sockeye salmon, the brain parasite prevalence data and biochemical genetic data are much less variable from year to year than the scale pattern data.

In the third scenario, we examined the reliability of stock composition estimates as a function of mixture sample size for cases where a single stock, several stocks, or all stocks contribute to the mixture sample. This generalization is important since it is seldom known a priori how many stocks are exploited in a particular mixed-stock fishery.

Methods

Data Sources

Since 1982, researchers with the Alaska Department of Fish and Game (ADFG), the Canada Department of Fisheries and Oceans (DFO), the National Marine Fisheries Service (NMFS), and the Pacific Salmon Commission (PSC) have collected extensive data on characteristics of sockeye salmon populations in British Columbia and Southeast Alaska. We rely on these data to demonstrate the relative utility of the various markers and statistical procedures for stock identification. Although little of this information has been published to date, summaries of data available are provided in appendices to Wood et al. (1988). Published sources include Utter et al. (1984); Bailey and Margolis (1987); Winans and Helle (1989); Moles et al. (1989); and various reports in the ADFG Technical Report Series; additional data are available from unpublished reports to the PSC and from unpublished files of the four agencies involved. We refer to the work of individual investigators and agencies as appropriate in the text. General descriptions of sampling and analytical procedures for data collection can be found in Wood et al. (1987c).

Biological Markers

The biological markers evaluated in this report include freshwater age, six scale pattern variables, the prevalence of the brain parasite *Myxobolus neurobius*, and five biochemical genetic traits. Freshwater and total ages were determined from scales and in some samples, also from the surface of otoliths as described by Bilton and Jenkinson (1968). Six scale pattern variables were considered but only for the dominant age class in the samples (age 1.3 or 5_2). These included the number of circuli (NC_i) and incremental distances or widths (ID_i) within each of three growth zones representing the first year's growth in freshwater ($i = 1$), "spring" or "plus" growth in freshwater during the second year prior to entering the sea ($i = 2$), and marine growth during the remainder of the second year ($i = 3$) (after Marshall et al. 1984). The prevalence of the brain parasite *Myxobolus neurobius* was determined using the procedure recommended by Dr. L. Margolis (Pacific Biological Station, Nanaimo, B.C., V9R 5K6) and described briefly by Quinn et al. (1987). Parasite prevalence refers to the proportion of brain samples infected by the parasite. Electrophoretic variation was considered at five loci: phosphoglucomutase-1,2 (*Pgm*-1 and *Pgm*-2), lactate dehydrogenase-4 (*Ldh*-4), and isocitrate dehydrogenase-3,4 (*Idh*-3 and *Idh*-4). Standard techniques were used to assess electrophoretic variation (e.g. Utter et al. 1984).

Reference Data

Reference data on the biological markers described above have been collected from at least 51 principal sockeye popu-

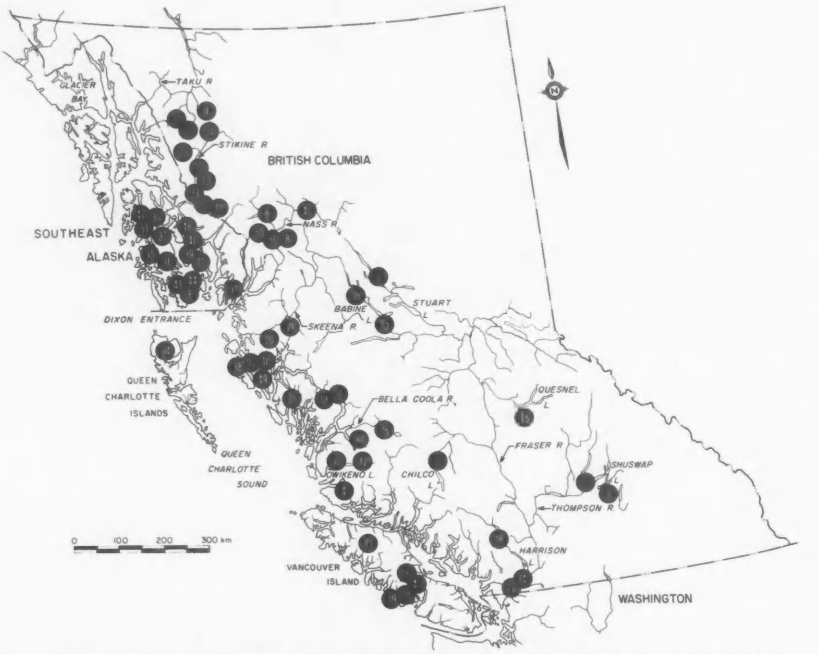


FIG. 1. Sockeye salmon populations in northern British Columbia and Southeast Alaska included in the reference samples. Numbers relate to names listed in Fig. 2.

lations in British Columbia and Southeast Alaska situated between the Fraser and Taku Rivers (Fig. 1 and 2). To estimate stock composition in samples from mixed-stock fisheries, the reference data for each marker in each stock must be expressed as a probability distribution defining the probability of choosing a fish with a particular set of characteristics, for example, freshwater age 2. The maximum likelihood model formulated by Fournier et al. (1984) and used here to estimate stock composition, requires that all markers considered in the analysis be independent of one another. The six scale pattern variables are highly correlated whereas the freshwater age, brain parasite prevalence, and electrophoretic traits are not, and can be assumed to be independent. Accordingly, principal components analysis was performed to express most of the information contained in the six scale pattern variables in three uncorrelated scale factors (e.g. Messinger and Bilton 1974). Only the first three scale factors were used because they accounted for over 90% of the variance in the six scale variables in the reference samples. These factors were defined as:

$$(1)\quad \text{factor } 1 = 0.032\ \mathrm{SNC}_1 + 0.041\ \mathrm{SID}_1 + 0.521\ \mathrm{SNC}_2 + 0.513\ \mathrm{SID}_2 + 0.019\ \mathrm{SNC}_3 + 0.085\ \mathrm{SID}_3$$

$$(2)\quad \text{factor } 2 = 0.524\ \mathrm{SNC}_1 + 0.519\ \mathrm{SID}_1 + 0.040\ \mathrm{SNC}_2 + 0.032\ \mathrm{SID}_2 - 0.028\ \mathrm{SNC}_3 + 0.100\ \mathrm{SID}_3$$

$$(3)\quad \text{factor } 3 = 0.074\ \mathrm{SNC}_1 + 0.002\ \mathrm{SID}_1 + 0.066\ \mathrm{SNC}_2 + 0.035\ \mathrm{SID}_2 + 0.529\ \mathrm{SNC}_3 + 0.572\ \mathrm{SID}_3$$

where SNC_i and SID_i are standardized values for NC_i and ID_i obtained by subtracting the corresponding mean value across all stocks, and then dividing by the standard deviation across all stocks. These scale factors are continuous variables and appproximately normally distributed, with means in the stocks examined ranging from -1.082 to 1.459, -1.722 to 1.180, and -0.967 to 1.725, for factors 1, 2, and 3, respectively. To facilitate mixture analysis with existing computer programs, the normal distributions specified by the mean and variance of each scale factor in each stock were represented as 15-bin histograms (i.e. discrete frequency data). Genotypic frequencies were calculated from multinomial distributions (assuming Hardy-Weinberg equilibrium within stocks) specified by the observed allele frequencies. Observed freshwater age composition and brain parasite prevalence data were summarized as four-bin and two-bin histograms, respectively.

Monte Carlo Simulations

General Procedures

The Monte Carlo simulations described in this paper follow the general procedures outlined by Wood et al. (1987a). The reference probability distributions characterizing each stock were considered to represent the true (population) distributions for that stock. Independent samples were drawn randomly with replacement from these distributions to generate a new "learning sample" of the same sample size. This step was required to reproduce the sampling error that actually occurs at the time the real stock is sampled. The new learning sample was then "smoothed" by calculating the corresponding discrete representations of normal distributions (for the scale data) or multinomial distributions (for the genetic data) as before using means and variances, or allele frequencies, respectively, calculated from the new learning samples. These smoothed learning samples were used to calculate the relative frequencies of multiple character combinations in each stock as required for the maximum likelihood analysis.

Stock composition ($\mathbf{p} = \{p_1, p_2, \ldots, p_n\}$ where p_i denotes the proportion of the mixture contributed by stock i) of simu-

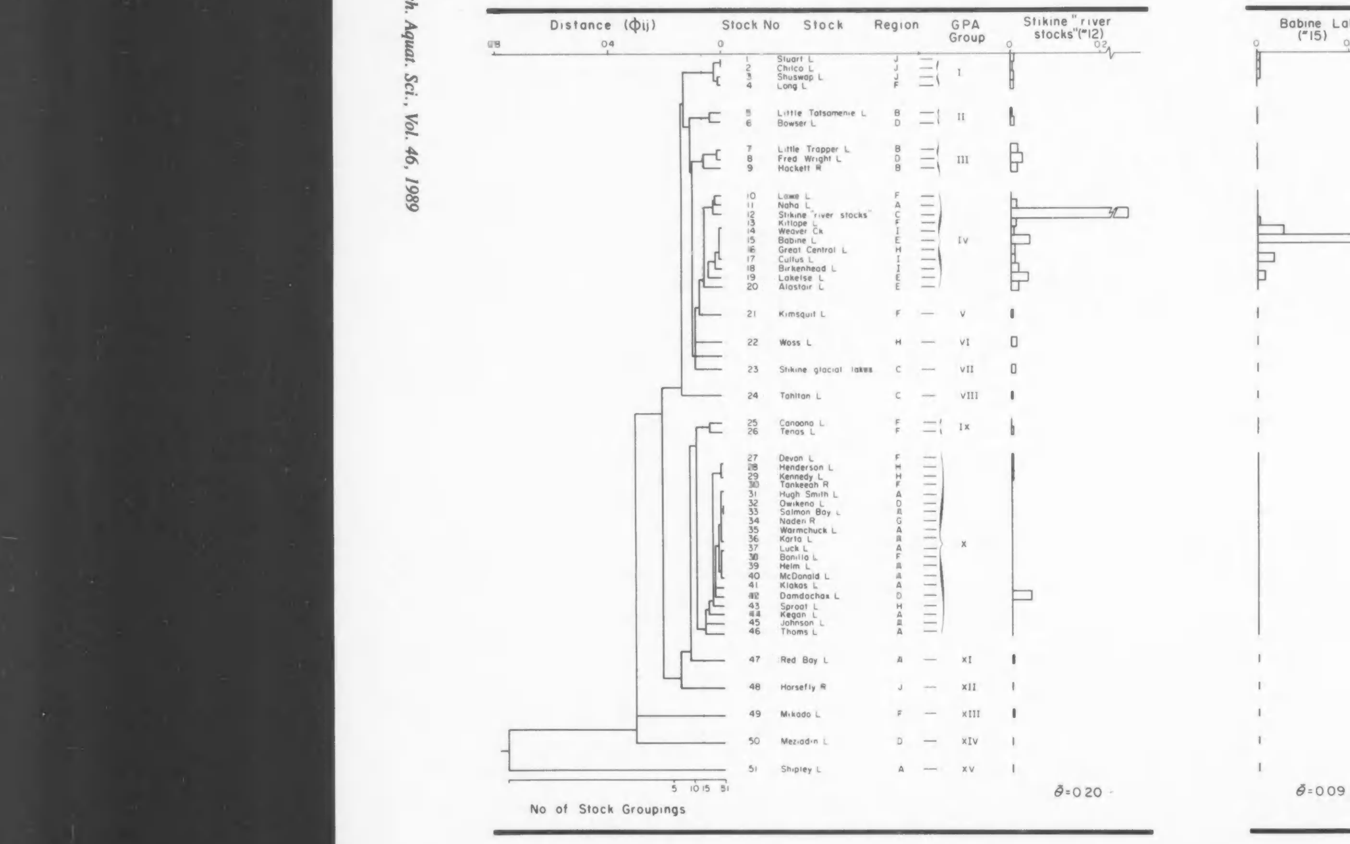
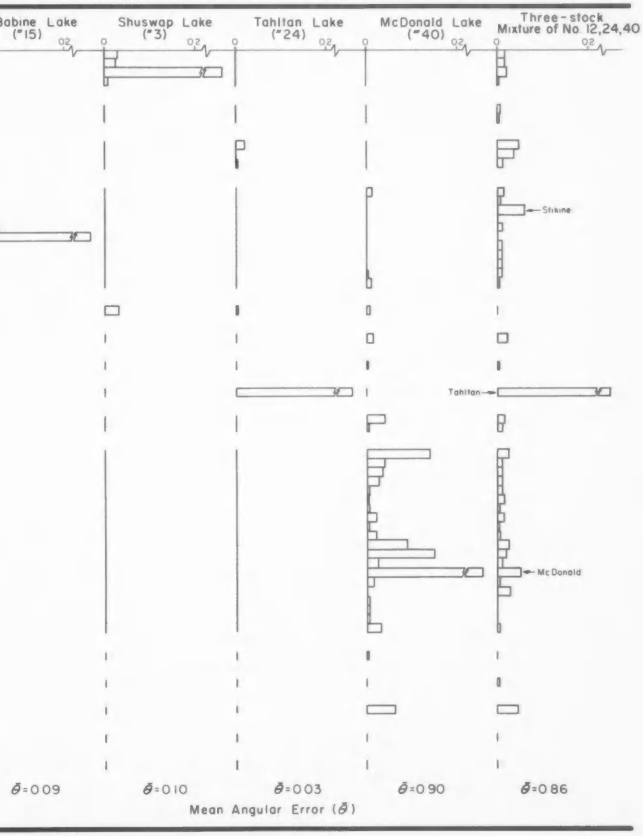


FIG. 2. Similarity dendrogram for 51 sockeye populations based on genetic, brain para
numerals represent stock-groupings formed to the left of $\phi_{ij} = 0.06$. Regions are defined
watershed; (C) Stikine watershed; (D) Nass watershed; (E) Skeena watershed; (F) Britis
Queen Charlotte Islands; (H) Vancouver Island; (I) lower Fraser watershed; and (J) upper F
mean estimated proportions (top scale, truncated at 0.2) for each stock for six simulated
stock (No. 12, 15, 3, 24, and 40, respectively) and the last comprised three stocks in equ



ain parasite, and freshwater age markers (set GPA). Roman
defined as: (A) Southeast Alaska Districts 101–111; (B) Taku
F) British Columbia north and central coast Areas 5–12; (G)
upper Fraser watershed, above Hell's Gate. Histograms show
simulated mixtures; the first five mixtures comprised a single
ks in equal proportions (No. 12, 24, and 40).

lated mixture samples was held constant in each example, referred to as a mixture "type." One hundred mixtures samples of each type were generated by selecting fish randomly with replacement according to the probability distributions specified in the reference data (not the learning samples). This procedure ensured that any error introduced by sampling the mixture was independent of that introduced while obtaining the learning samples. Thus, within each mixture type, mixture samples differed only because of random sampling of phenotypes, not because of differences in stock composition. We deliberately focussed attention on errors associated with estimating the stock composition of a mixture sample, not on statistical errors while inferring the stock composition of total catches from samples.

Seven different combinations of markers ("marker sets") were used to estimate the stock composition of the simulated mixture samples. The following abbreviations are used to simplify presentation: G denotes the five genetic (electrophoretic) markers; P, the brain parasite; A, freshwater age; and S, the scale pattern factors (for age 1.3 fish only). In these symbols, the seven marker sets are G, GP, GPA, GPAS, S, SP, and SPA. No other combinations could be evaluated without drastically reducing the number of stocks considered in the analysis since only two and four observable states are associated with marker sets P and A, respectively; that is, the problem is unidentifiable if more than two or four stocks, respectively, are considered in the analysis.

Marker set S is available for age 1.3 fish only, and this age group occurs only rarely in nine of the 51 stocks. Thus, the 51-stock mixture problem is unidentifiable using marker set S, SP, or SPA because only marker sets P and PA are available for other age classes of fish in the mixture sample.(Note that the 51-stock problem remains identifiable using marker set GPAS despite the occurrence of multiple age classes in the mixture because many observable states are associated with marker set G). For this reason, a subset of only 42 stocks was considered in the analyses with marker sets S, SP, and SPA. New mixture samples were generated as before except that only age 1.3 fish were selected. Estimates from the 51-stock problems using marker sets G, GP, GPA, and GPAS are directly comparable because identical mixture samples were analyzed; similarly, estimates from the 42-stock problems using marker sets S, SP, and SPA are directly comparable. Mixture samples used in the 51- and 42-stock problems will differ slightly due to random sampling error and to the exclusion of age classes other than age 1.3 in the latter mixture samples.

Mixture composition (**p**) was estimated using the maximum likelihood method of Fournier et al. (1984) and conditional probabilities calculated from the smoothed learning samples for each mixture sample and each marker set. For example, under the first scenario (ideal conditions), this resulted in a total of 3500 estimates (five mixture types × 100 mixture samples/type × seven marker sets). Means and standard deviations were calculated from the 100 estimates for each combination of mixture type and marker set. We refer to the difference between the mean estimate of the proportion contributed by a selected stock and its true contribution (1.000) as the "mean error" for that stock. The mean error and standard deviation statistics reflect bias and imprecision, respectively, for estimates of mixing proportions of particular stocks ($\hat{p}_i$). They also provide a convenient measure of the overall performance of the estimation procedure when the mixture consists entirely of one stock. However, overall performance is more difficult to characterize when more than one stock contributes to the mixture sample; for this purpose, we use the mean angular deviation ($\bar{\Theta}$) of the estimated vector of stock proportions ($\hat{\mathbf{p}}$) from the true vector (**p**). Mean angular deviation reflects the combined effects of bias and imprecision (see Wood et al. 1987a).

Accuracy and precision of the stock composition estimates can be improved by reducing the number of individual stocks being considered, thereby simplifying the mixture problem. We used the allocate-sum procedure of Wood et al. (1987a) in which the collective contribution by a group of stocks is determined by summing the allocations to the individual stocks obtained in the 51-stock (or 42-stock) analysis. Standard deviations are then recalculated for the 100 summed estimates. We defined stock-groupings from similarity dendrograms as described by Wood et al. (1987a) and Wood (1989) (e.g. Fig. 2). We grouped stocks according to various "distance thresholds" which we selected to compare the accuracy and precision of estimates for the 51 (or 42) individual stocks with that of summed estimates for 30, 20, 10, and five stock-groupings, respectively.

Simulations under ideal conditions

Five mixture types of known composition (**p**) were analyzed to evaluate the reliability of stock composition estimates under ideal conditions. Each mixture sample included 300 fish from a single stock to highlight potential misclassifications. The contributing stocks were selected to provide a range from easy to difficult mixture problems as judged from the similarity dendrogram in Fig. 2. Mixture 1 included only fish from the river-rearing Stikine "river stocks"; mixture 2, from Babine Lake; mixture 3, from Shuswap Lake; mixture 4, from Tahltan Lake; and mixture 5, from McDonald Lake.

Simulations with scale pattern variability

The five mixture types analyzed under ideal conditions were reanalyzed using marker sets S, SP, SPA, and SPAG and learning samples which had been corrupted to simulate the annual variability expected for scale pattern variables. In these simulations, we assumed that scale factors are normally distributed within a population and that the mean of this distribution changes randomly from year to year whereas the variance remains constant. We also assumed that the variance of the means over time for each scale factor is identical for all stocks. These assumptions are not entirely realistic because the variances of scale pattern variables do vary among stocks and from year to year in some stocks (Wood et al. 1988). Nevertheless, some simplification and generalization is necessary because empirical data on the extent of variation in scale factors are not available for all stocks. These assumptions make it possible to assess, in a simple way, whether the results of our stock composition analyses are sensitive to annual variations in scale patterns.

An error term (ϵ_{AV}) simulating annual variation in the mean of each scale factor was drawn randomly and independently for each stock from a normal distribution with a mean of zero and a standard deviation of 0.5, 0.6, and 0.7 for scale factors 1, 2, and 3, respectively. These standard deviations were based on preliminary estimates from empirical data on annual variation in 16 of the 42 stocks in the analysis. (A similar, but revised analysis of these data is described in the next paragraph.) The error terms were added directly to the scale factor means in each learning sample before calculating the expected normal distribution for the smoothed learning samples. Thus, error terms were applied only to the learning samples, not to the mixture samples. This procedure simulated the analysis of con-

TABLE 1. Summary of variation in scale factors observed (obs.) in samples collected in different years for 16 stocks in Alaska and British Columbia. Deviations of repeat sample means from the overall mean (data pooled over years) are compared with deviations expected (expect.) if variation were caused only by resampling, not by annual changes. Expected deviations were computed by resampling techniques as described in Table 2.

			Factor 1				Factor 2				Factor 3			
					Deviations				Deviations				Deviations	
Stock	Year	Sample size	Mean	SD	Obs.	Expect.	Mean	SD	Obs.	Expect.	Mean	SD	Obs.	Expect.
Alaska														
McDonald	1982	100	−0.76	0.23	0.27	−0.01	−1.29	0.50	0.19	−0.02	0.73	0.72	0.04	−0.07
	1983	10	−0.58		0.09	−0.13	−0.69		−0.41	−0.05	0.40		0.37	−0.01
	1984	20	−0.83	0.15	0.34	−0.14	−1.03	0.28	−0.07	0.06	0.75	0.79	0.02	0.24
	1985	10	0.27		−0.76	−0.16	−1.25		0.15	−0.12	0.68		0.09	−0.01
	1986	100	−0.22	0.56	−0.27	0.01	−0.94	0.57	−0.15	0.08	0.85	0.73	−0.09	0.07
Naha	1982	100	−0.90	0.30	0.26	0.02	−0.25	0.56	0.30	0.07	0.08	0.65	0.22	0.02
	1983	10	−0.32		−0.32	−0.07	0.49		−0.44	0.21	1.18		−0.88	−0.10
	1984	10	−0.93	0.12	0.29	0.09	0.57	0.40	−0.52	−0.22	0.33	0.22	−0.03	−0.31
	1985	10	0.04		−0.68	0.01	0.69		−0.64	−0.03	−0.10		0.40	0.13
	1986	20	0.29	0.84	−1.48	−0.03	0.75	0.50	−0.70	0.00	1.12	0.66	−0.82	0.22
Helm	1982	36	1.39	0.71	−0.01	0.12	0.76	0.62	0.14	0.12	0.75	0.99	1.64	0.07
	1983	11	0.34		1.05	−0.05	0.40		0.51	0.04		0.82	1.57	0.13
	1985	10	3.47		−2.08	0.16	2.87		−1.96	−0.06	14.32		−11.93	0.12
	1986	20	0.91	0.39	0.48	0.03	0.46	0.35	0.44	0.07	0.24	0.86	2.15	0.36
Johnson	1982	100	0.36	0.67	−0.20	−0.16	0.23	0.66	0.12	0.05	0.67	0.66	0.03	0.07
	1983	10	−0.58		0.74	0.06	0.81		−0.46	−0.16	1.06		−0.36	−0.09
	1984	20	−0.92	0.14	1.07	0.01	0.57	0.83	−0.21	0.02	0.57	0.56	0.13	−0.10
	1985	10	0.53		−0.37	−0.11	0.39		−0.04	−0.04	0.33		0.37	0.08
	1986	6	0.93	0.71	−0.77	0.12	0.88	0.22	−0.52	0.54	1.73	0.17	−1.03	−0.25
Kegan	1982	100	−0.68	0.32	0.03	0.06	−0.15	0.38	−0.21	−0.04	0.88	0.72	0.14	−0.02
	1983	10	−0.69		0.04	−0.10	−2.15		1.79	0.07	1.55		−0.53	−0.22
	1984	20	−0.83	0.32	0.18	0.01	−0.62	0.55	0.27	0.03	1.00	0.64	0.01	0.03
	1985	10	−0.01		−0.64	0.17	−0.18		−0.18	−0.05	1.53		−0.51	−0.22
	1986	10	−0.59		−0.06	0.01	−0.31		−0.05	−0.10	1.39		−0.37	0.25
Klakas	1982	100	1.28	0.97	−0.58	0.14	−1.34	0.43	−0.04	−0.08	0.48	1.05	0.03	0.08
	1983	10	−0.49		1.20	0.24	−0.84		−0.55	−0.01	1.00		−0.48	0.67
	1984	20	−1.08	0.26	1.79	−0.27	−1.72	0.42	0.34	0.00	0.04	0.67	0.47	0.07
	1985	10	−0.29		1.00	−0.10	−1.68		0.29	0.05	1.29		−0.77	0.08
	1986													
Warm Chuck	1982	68	−0.66	0.29	0.10	−1.10	1.18	0.72	−0.20	0.01	0.00	0.90	0.26	−0.04
	1983	10	−0.61		0.05	0.06	1.05		−0.07	−0.10	0.69		−0.43	0.19
	1984	18	−0.81	0.23	0.25	0.10	0.52	0.53	0.46	0.24	0.11	0.91	0.15	0.20
	1985	10	−0.03		−0.53	0.13	1.16		−0.18	0.31	0.94		−0.68	0.15
	1986	20	−0.24	0.49	−0.32	−0.06	0.59	0.53	0.39	−0.01	0.73	0.48	−0.47	0.39
Luck	1982	50	−0.63		0.44	0.05	0.02		−0.12	−0.02	0.70		0.15	−0.08
	1983	10	−0.77		0.58	0.41	−0.06		−0.04	−0.29	1.30		−0.45	0.13
	1984	20	−0.75	0.12	0.56	0.01	0.00	0.32	−0.11	−0.01	0.57	0.71	0.28	−0.06
	1985	10	0.41		−0.60	0.24	−0.05		−0.05	0.12	0.67		0.18	0.03
	1986	20	1.46	0.86	−1.65	0.11	−0.56	0.45	0.46	−0.03	1.37	0.78	−0.52	−0.20
Salmon Bay	1982	100	−0.71	0.17	0.32	0.08	−0.56	0.59	0.11	0.09	0.70	0.68	−0.02	0.06
	1983	20	−0.44	0.52	0.05	0.12	−0.04	0.50	−0.40	−0.05	0.43	0.61	0.25	−0.24
	1984	10	−0.44		0.05	0.04	−0.04		−0.41	−0.27	0.43		0.25	−0.10
	1985	10	0.40		−0.79	−0.32	−0.50		0.05	−0.36	0.68	0.79	0.00	−0.19
	1986	20	0.92	0.72	−1.30	−0.07	−0.46	0.73	0.01	−0.16	0.93		−0.25	−0.07
Red Bay	1982	106	−0.87	0. 27	0.08	0.03	0.81	0.53	−0.42	0.05	−0.05	0.63	−0.06	0.05
	1983	10	−0.94		0.15	0.12	−0.91		1.30	−0.06	0.07		−0.18	0.29
	1984	0	−0.67		−0.12	0.04	−1.15		1.54	−0.07	−0.58		0.47	0.05
	1985	10	−0.68		−0.11	0.07	0.01		0.38	−0.08	−0.67		0.56	−0.12
	1986	20	−0.41	0.48	−0.38	−0.01	−0.24	0.48	0.63	−0.01	−0.01	0.81	−0.10	0.18

TABLE 1. (*continued*)

Thoms	1982	100	−0.86	0.22	0.12	0.00	1.03	0.56	−0.11	0.02	−0.23	1.78	0.08	−0.04
	1983	10	−0.50		−0.24	0.13	0.65		0.27	0.15	−0.06		−0.09	−0.57
	1984	20	−0.72	0.23	−0.02	0.04	0.63	0.57	0.29	−0.07	0.16	.91	−0.31	0.25
	1985	10	−0.13		−0.61	−0.01	0.75		0.17	−0.09	−0.30		0.15	−0.30
	1986	4	−0.06	0.23	−0.68	0.04	0.81	0.57	0.11	0.23	0.36	0.57	−0.51	0.70
British Columbia														
Tahltan	1982	139	0.68		−0.69	0.01	0.76		−0.42	−0.01	0.76		−0.95	−0.05
	1983	111	0.26		−0.27	0.08	0.45		−0.11	0.01	0.45		−0.64	−0.02
	1984	44	−0.60	0.39	0.59	−0.05	−0.02	0.40	0.36	0.02	−0.85	0.74	0.66	0.11
	1985	18	−0.54	0.42	0.53	0.10	0.04	0.49	0.30	0.12	−1.27	0.54	1.08	0.10
	1986	199	−0.46	0.48	0.45	0.07	0.09	0.50	0.25	0.01	−0.96	0.71	0.78	−0.10
Chutine Lake	1984	15	−0.39	0.74	0.57	0.13	−1.08	0.63	−0.04	0.27	0.16	1.01	−0.10	−0.24
	1985	7	1.39	1.70	−1.21	0.82	−1.20	0.73	0.08	−0.17	−0.14	0.90	0.20	−0.03
Chutine River	1984	36	0.04	1.29	0.10	0.10	−0.86	0.89	−0.05	−0.05	−0.28	1.03	0.08	0.14
	1985	16	0.36	0.87	−0.22	0.37	−1.01	0.75	0.11	0.17	−0.02	0.67	−0.18	−0.02
Skud	1984	18	0.34	0.82	−0.26	−0.04	−0.85	0.60	0.12	0.04	0.08	0.79	−0.16	0.05
	1985	39	−0.04	0.74	0.12	0.17	−0.67	0.77	−0.06	0.02	−0.15	0.71	0.07	0.03
Iskut	1984	70	0.40	1.17	−0.20	−0.03	−0.82	0.59	0.06	−0.03	0.34	1.10	−0.09	−0.22
	1985	197	0.14	0.83	0.07	0.04	−0.74	0.63	−0.02	0.04	0.21	0.89	0.03	0.08

temporary mixture samples using historical learning samples as required for most analyses during the fishing season.

To ensure that our simulated variability corresponded approximately to levels observed in nature, we first estimated the extent of annual variability in scale pattern data from a subset of 16 of the 42 stocks for which scale pattern data for age 1.3 sockeye are available in more than one year. Means for each scale factor in each stock were calculated (Table 1) for the basic scale variables summarized by Wood et al. (1988) using equations (1) to (3). It was not possible to compute the corresponding variances for these scale factors from the published data because the six basic scale variables are highly correlated. However, scale factor variances were calculated from unpublished data (Glen Oliver, ADFG, P.O. Box 3-2000, Juneau, AK 99802, pers. comm; and C. C. Wood) available for most but not all of the samples summarized by Wood et al. (1988). Because scale samples were not collected in the same years for all stocks, sample sizes were usually small, and scale factor variances were not always homogeneous, we were unable to obtain meaningful estimates of the variance due to year effects by an analysis of variance with the smaller, unpublished data set. Instead, we examined variation in the scale factor means within the larger, published data set, ignoring differences in sample size, and computed approximate statistics analogous to the standard deviations of scale factor means for each stock. In general, the variation in scale factor means will depend on sample size, but this procedure is adequate for determining trial values for the error term (ϵ_{AV}) used with typical sample sizes in the simulations where the effect of sample size was also ignored. First, we calculated a pooled mean (the weighted average of the sample means in different years) and a pooled estimate of the within-year variance (Snedecor and Cochran 1980, p. 91) for each scale factor (i) in each stock (j) using all available data (Table 2). Next, we calculated deviations of the sample means in each year from the pooled mean (referred to as the "observed deviations"). The sum of the squared observed deviations, divided by one less than the number of sample years (the mean sum of squares, denoted t_{ij}^2) is an approximate estimate of the total variance of the sample means for a typical sample size, where variation arises from the combined effects of random resampling and annual changes in scale patterns. To determine the effect of random resampling alone, we generated new samples of identical sizes in each year, by drawing randomly from the normal distribution specified by the pooled mean and variance characteristic of each scale factor in each stock. The deviations of the means of the simulated samples from the pooled mean are referred to as the "resampled deviations." The mean sum of squares of the resampled deviations (r_{ij}^2) was calculated as for the mean sum of squares of the observed deviations (t_{ij}^2). Finally, we estimated the component of variance of the mean due only to annual variation by subtraction ($s_{AVij}^2 = t_{ij}^2 - r_{ij}^2$). This step was necessary to avoid introducing sampling error twice in the simulations. In most cases, t_{ij}^2 far exceeded r_{ij}^2. The estimated standard deviations of the sample means due to annual effects (s_{AVij}) varied among stocks from 0 to 1.4, 0 to 1.2, and 0 to 7.1 for scale factors 1, 2, and 3, respectively (Fig. 3).

Simulations with reduced mixture sample size

We repeated simulations with mixture types 1, 4, and 5 (i.e. 100% mainstem Stikine River, Tahltan and McDonald Lakes, respectively) using mixture sample sizes of 11, 33, 100, and 300 to determine how large samples must be to ensure useful estimates of stock composition. In addition, we assessed the accuracy of estimates for mixtures comprising more than one stock by simulating two other mixture types comprising equal proportions of three stocks (the mainstem Stikine River, Tahltan, and McDonald Lakes, mixture 6) and from all 51 stocks (mixture 7). Mixture 6 was selected as a practical example since all three stocks are caught in mixed-stock fisheries in Southeast Alaska. Mixture 6 was analyzed using sample sizes of 33, 99, and 300 (i.e. 11, 33, and 100 fish per stock) and mixture 7, using sample sizes of 51, 102, 153, and 306 (i.e. 1, 2, 3, and 6 fish per stock).

Results

Stock Composition Analysis under Ideal Conditions

Simulations with the maximum likelihood mixture model demonstrate that biological markers can be used to differentiate most of the 51 sockeye populations reliably under ideal conditions; that is, where (1) all reference samples are perfectly representative, (2) genotype frequencies are in Hardy-Weinberg equilibrium, (3) scale pattern factors are normally distributed, (4) mixture samples are relatively large ($n=300$), and (5) only stocks represented by reference samples contribute to the mixture samples. Accuracy and precision are also improved by reducing the complexity of the mixture problem by grouping similar stocks together using the allocate-sum procedure. This procedure works because estimated mixing proportions tend to be most biased within clusters of similar stocks, whereas the collective contribution of the cluster is more accurately determined (Fig. 2; also Wood 1989). Sometimes this grouping procedure improves the reliability of estimates to a greater extent for one marker set than for other, larger marker sets (e.g. Fig. 4A, mixture 1, SP versus SPA). Presumably this occurs because different similarity dendrograms are used to derive the summing rules for each marker set.

The accuracy and precision of stock composition estimates increases as additional markers are considered in the analyses (Fig. 4). The only two exceptions to this pattern occur when estimates are already very accurate (Fig. 4A, mixtures 4 and 5). These anomalies are attributed to slight errors in solving the model equations by iteration using a minimization algorithm (Wood et al. 1987a). In two other cases, precision appears to deteriorate as additional markers are considered, but in fact, this results from an improvement in accuracy such that the mean error is reduced towards a value of ±50% at which point the variance of a proportion is maximal (Fig. 4B, mixtures 3 and 5).

A general feature of these results is that some marker sets serve to distinguish some stocks very reliably but not others. For example, the scale pattern factors provide very poor estimates for the Shuswap Lake mixture (Fig. 4, mixture 3) but extremely good estimates for the McDonald Lake mixture (Fig. 4, mixture 5).

Of the five examples analyzed, the river-rearing sockeye of the Stikine are the most difficult stock to distinguish (Fig. 4, mixture 1). This stock is not genetically distinct and scale pattern factors alone are of limited utility; only the marker sets SP, SPA, and GPAS come close to giving acceptable estimates with a bias less than 15% and 95% confidence interval of roughly ±10–15%.

In contrast, the Tahltan Lake stock is distinguished reliably by most marker sets (Fig. 4, mixture 4). Tahltan Lake sockeye are similar to Meziadin Lake sockeye using only marker set G or GP which accounts for the significant improvement in estimates at the 30-stock grouping level where contributions from these stocks are estimated collectively. Similarly, Tahltan and Meziadin stocks differ greatly in freshwater age composition and scale patterns so that adding either of these markers greatly improves the estimates.

The Shuswap Lake stock is distinguished fairly well by the combinations GPA and GPAS (bias <10–15%, 95% confidence interval ≃20%) but poorly by any other combinations (Fig. 4, mixture 3). This stock is difficult to distinguish from other upper Fraser River stocks excluding the Horsefly River stock, and estimates are much more reliable (bias <5%, 95% confidence interval ≃10%) when these stocks are considered together as one stock–group (the 30-stock grouping corresponding to group I in Fig. 2).

The McDonald Lake stock is very difficult to distinguish from other coastal lake stocks using any marker other than scale pattern factors (Fig. 4, mixture 5). Because many such coastal

TABLE 2. Method for estimating annual variation of mean scale factors from empirical data. Similar calculations were performed for factors 2 and 3, and the other 15 stocks in Table 1.

			Factor 1				
			Observed			Resampled	
Stock	Year	Sample size	Mean	SD	Deviation[b]	Mean[c]	Deviation
McDonald Lake	1982	100	−0.76	0.23	0.27	−0.48	−0.01
	1983	10	−0.58		0.09	−0.36	−0.13
	1984	20	−0.83	0.15	0.34	−0.36	−0.14
	1985	10	0.27		−0.76	−0.33	−0.16
	1986	100	−0.22	0.56	−0.27	−0.50	0.01
Pooled data		240	−0.49	0.54		−0.47	
Pooled SD[a]		220		0.41			
Mean sum of squared deviations[d]			$t^2=0.212$		$r^2=0.016$		
Approximate SD of sample means associated only with annual variation in scale factors			$s_{AV}=\sqrt{(t^2-r^2)}=0.44$				

[a] Pooled estimate of the within-year standard deviation based on the individual estimates in 1982, 1984, and 1986.

[b] Deviations of means of annual samples from the overall mean (−0.49) obtained by pooling data over all years.

[c] Resampled means computed by drawing independent, random samples of 100 for 1982, 10 for 1983, etc. from a normal distribution with a mean of −0.49 and a standard deviation of 0.41 based on the pooled estimates from all available data.

[d] Mean sum of squared deviations of observed means is denoted t^2 since this reflects total variation, i.e. both resampling and annual variation; the mean sum of squared deviations for resampled means is denoted r^2. In each case, the sum of squared deviations is divided by the number of years minus one.

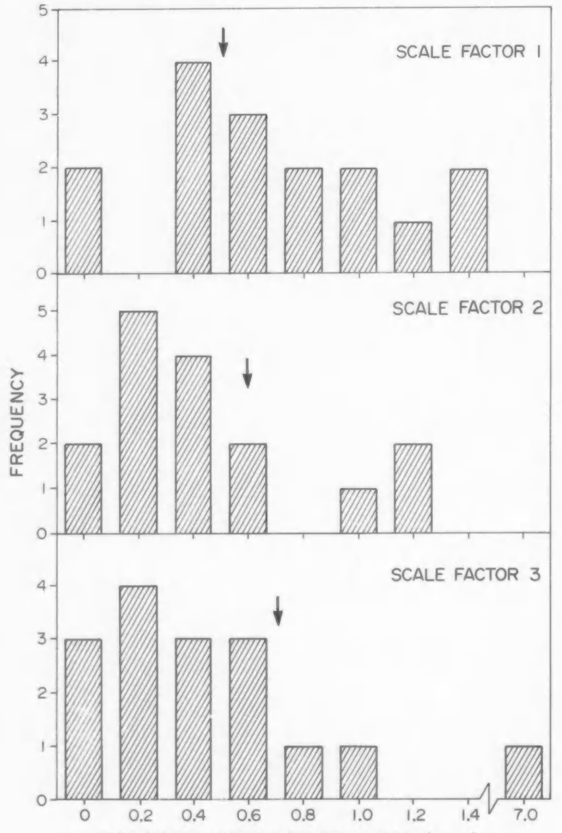


FIG. 3. Frequency distributions of estimated standard deviations of scale factor means due to annual variation (s_{AVij}) in 16 stocks for which samples are available in more than one year. Arrows indicate trial values applied to all 42 stocks in the simulations.

stocks are considered in this analysis, with varying degrees of similarity, extensive grouping is required to produce acceptable estimates using the nonscale pattern markers.

Bias from Annual Variation in Biological Markers

We examined scale pattern data presented in Table 1 and data summarized by Wood et al. (1988) to determine whether phenotypic frequencies for the biological markers considered here change significantly from year to year. The number of stocks showing significant annual variation ($p<0.05$, t-test corrected for multiple comparisons within stocks) is summarized in Table 3 for each type of marker according to the number of years available to test for differences. Note that the opportunity to detect significant annual variation between any two years increases as more years are compared.

The comparisons in Table 3 indicate that scale factors, and to a lesser degree freshwater age composition, exhibit detectable annual variation whereas the genetic and brain parasite markers do not. Significant differences between samples from the same stock in different years can be detected in 10, 8, and 4 out of 16 stocks for scale factors 1, 2 and 3, respectively. For some stocks, annual variation in scale factors can be detected even where small samples (mean = 48.5 age 1.3 fish) were collected in only two consecutive years.

In contrast, no significant differences can be detected for any of the three genetic markers in 21 different stocks. Only one out of 23 stocks shows detectable annual variation in the prevalence of the brain parasite; this result would be expected from chance alone in 23 comparisons with $p=0.05$.

Annual variation in freshwater age can be detected in all 14 stocks sampled intensively (mean samples sizes >250 fish) for at least 4 yr. However, no annual variation can be detected in the three comparisons where sample sizes and the number of years examined corresponded to those for the genetic, parasite and scale pattern markers. Thus, we conclude that although freshwater age composition varies from year to year in many stocks, the magnitude of this variation is generally much less than that for scale pattern markers.

Annual variation in sockeye scale factors will result in biased estimates of stock composition because reference data collected from a stock in previous years will not be representative of that stock in the current year. To estimate the expected magnitude of bias due to annual variation in scale factors, we corrupted learning samples by adding an error term selected randomly from normal distributions with means of zero and standard deviations as calculated from empirical data for 16 stocks (see Methods). Using these corrupted learning samples, we repeated previous simulations with mixtures types 1–5 using marker sets S, SP, SPA, and SPAG for the 42 stocks for which age 1.3 scale pattern data are available. It is clear from our results that the observed level of annual variation in scale patterns can severely bias estimates of stock composition (Fig. 5). This additional source of bias was least evident where estimates were already poor even with representative reference samples. After simulating annual variation, mean error increased from 58.4 to 72.2% for mixture type 3 (an increase of only 13.8%) but from 0.2 to 55.5% for mixture type 4 (an increase of 55.3%) using marker sets.

Effect of Mixture Sample Size and Mixture Composition

The reliability of stock composition estimates using the GPA marker set decreases as mixture sample size decreases but at different rates depending on which stocks contributed to the mixture (Fig. 6). Whereas the estimates for mixture 4 (100% Tahltan Lake) are acceptable with samples as small as 11 fish, those for mixture 1 (100% Stikine river stocks) deteriorate markedly with sample sizes <100 fish. Mixture sample size has only a slight effect on the reliability of estimates for mixture 5 (100% McDonald Lake) because these estimates are very unreliable at all sample sizes examined.

The mean angular error index ($\overline{\Theta}$) reflects both accuracy and precision, and is a convenient measure of performance for problems where more than one stock contributes to the mixture sample. The mean angular error index for the pure stock mixtures 1, 4, and 5 (solid symbols) with $n=300$ can be compared directly with the more familiar proportion mean error in Fig. 4 for the corresponding analyses using the GPA marker set. Note that these maximum likelihood estimates of stock composition must converge to the correct solution ($\Theta=0.0$) as mixture sample sizes become infinitely large (Fournier et al. 1984); this fact was also demonstrated for the computer program used here in previous simulations by Wood et al. (1987a).

The reliability of stock composition estimates for mixtures of three and 51 stocks in equal proportions improves only

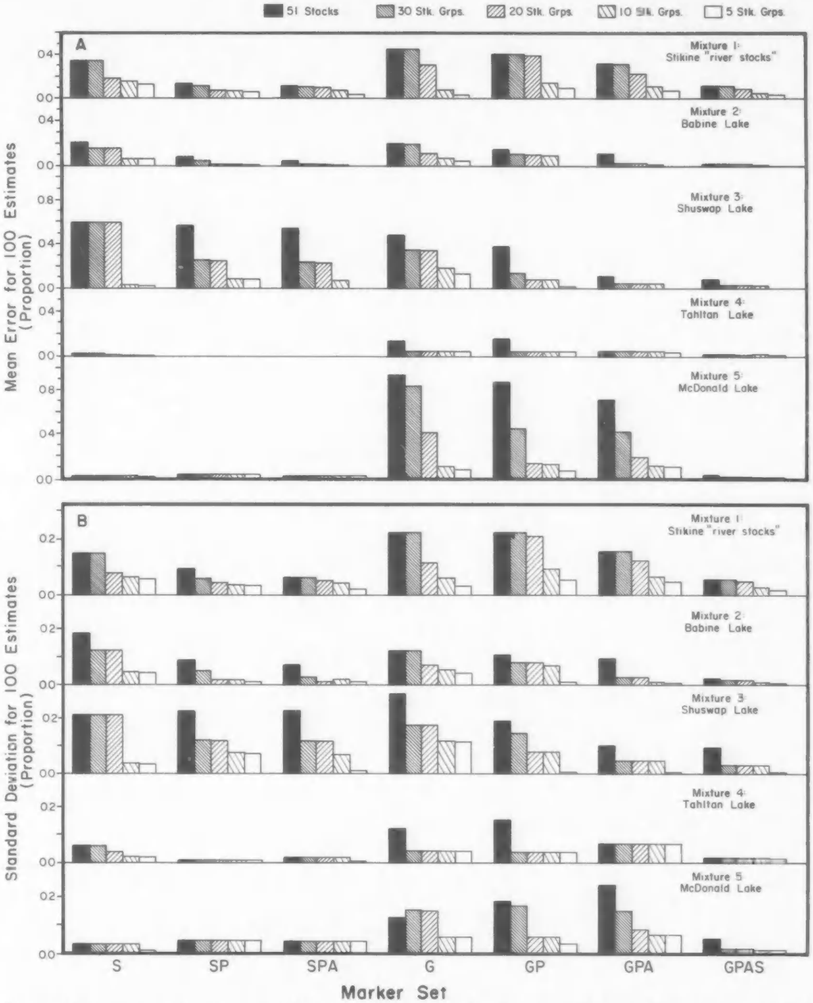


FIG. 4. Improvement in accuracy (A) and precision (B) of stock composition estimates by using additional biological markers in combination and by summing estimates within groupings of similar stocks for mixture types 1–5. Letters designate the marker sets used to estimate stock composition: S denotes the scale pattern factors (for age 1.3 fish only); P, the brain parasite; A, freshwater age; and G the five genetic (electrophoretic) markers.

slightly as mixture sample size is increased from 33 to 300 fish. In fact, mean angular errors for these mixtures are about as large as expected for the least identifiable stock present in the mixture (i.e. McDonald Lake in the three-stock mixture) for mixture samples sizes ⩾99 fish.

Discussion

The foregoing simulation results demonstrate that current techniques for sockeye salmon identification can be very effective in particular applications. The usefulness of the techniques depend upon which stocks or stock-groupings are to be identified. Although it is impossible to evaluate all possible scenarios, the reliability of stock composition estimates for particular stocks can be inferred by noting their relative similarity to other stocks in a similarity dendrogram and by comparing this relative indicator of performance to absolute levels of performance determined through simulation runs (Wood 1989). Our results demonstrate that a large number of markers used in combination is required to solve complex coast-wide mixture problems. However, we conclude that under ideal conditions, most if not all of the 51 stocks considered here could be identified accurately enough for management purposes using the GPAS marker set.

Scale patterns show the greatest discriminatory power of any single type of biological marker tested here. In fact, scale patterns might allow slightly better discrimination among stocks than indicated in our results for three reasons. First, we examined only six large-distance, direct scale measures. Additional discriminating power can sometimes be achieved by measuring the width of smaller zones on scales (e.g. consecutive triplets of circuli) (Davis et al. 1989). Second, we may have sacrificed

TABLE 3. Results of statistical comparisons of annual variation in biological markers used for stock composition analysis. Numbers refer to the number of stocks examined for annual variation and the number showing significant variation ($P<0.05$). Data from Wood et al. (1988).

	Number of Years Compared										
	2 yr		3 yr		4 yr		5 yr		Total		Mean sample size
Marker	Total	Significant	Total	Significant	Total	Significant	Total	Significant	Total	Significant	
Scale patterns											
Factor 1	7	4	9	6					16	10	48.5
Factor 2	7	4	9	4					16	8	48.5
Factor 3	7	1	9	3					16	4	48.5
Freshwater age	1	0	2	0	2	2	12	12	17	14	512.8
Brain parasite	11	0	12	1					23	1	85.0
Genetic markers											
PGM-1	15	0	5	0	1	0			21	0	103.0
PGM-2	15	0	5	0	1	0			21	0	103.0
LDH-4	15	0	5	0	1	0			21	0	103.0

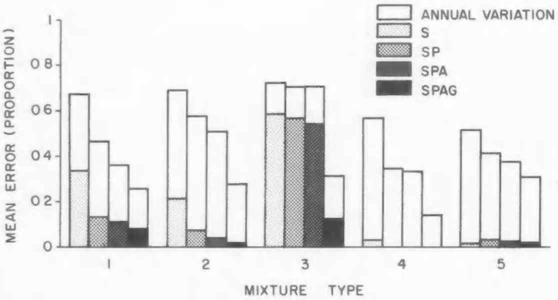


FIG. 5. Effect of simulated annual variation in scale pattern markers on mean error rates in estimated mixing proportions for the single contributing stock in mixture types 1–5. Stippled or solid segments of histograms for each marker set show error rate without annual variation; open segments show corresponding increment in error resulting from simulated annual variation.

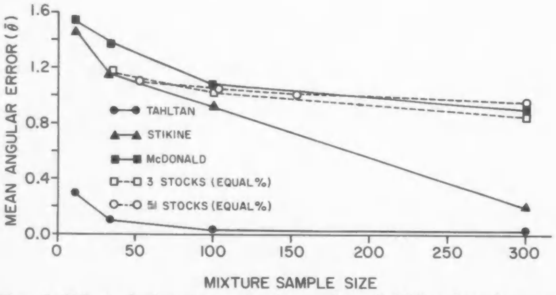


FIG. 6. Effect of mixture sample size on the reliability of stock composition estimates using the GPA marker set for mixtures comprising only a single stock (solid symbols) and mixtures comprising several or many stocks (open symbols).

some discriminatory power by converting continuous, and correlated scale variables into uncorrelated, normally-distributed scale factors, and then grouping each as a discrete variable. Owing to the small size of most reference samples available to us, some smoothing of empirical distributions is necessary, regardless of the variables selected, or the type of mixture analysis to be performed. Thus, the effect of representing continuous variables in discrete frequency distributions is probably negligible in view of the potential for error, common to all methods, in estimating the theoretical normal distributions from small samples; moreover, the number of possible states for the discrete variables is large (15). Third, we considered only one age class (age 1.3) which occurs most generally across the 51 stocks. It is conceivable, although improbable, that discrimination among some stocks would be better for other age classes.

Much of the discriminatory power of scale pattern factors results from the large number of observable states associated with each factor. This also implies that large sample sizes are required to correctly estimate the probability of observing each state in samples. We have attempted to minimize this problem by smoothing scale factor distributions in the learning samples, (i.e. by assuming they are normally distributed and estimating only two parameters, the mean and variance, for each factor in each stock). Mixture samples cannot be smoothed in the same way because mixtures of different normal distributions are not themselves normal distributions. Unfortunately, sampling error is usually more problematic in the mixture samples than in the learning samples owing to the smaller number of fish contributed from each stock represented in the mixture.

A more serious problem with scale pattern data is that they reflect conditions for juvenile growth (e.g. population density and climate), and often vary substantially from one year to the next (Table 1). Stock composition estimates obtained using learning samples in which scale data had been corrupted to mimic the effect of annual variation are seriously biased compared with those obtained using perfectly representative samples (Fig. 5). Of course, these simulations do not mimic the effects of annual variation exactly because too few data are available to calculate the extent of annual variation in nature. For example, in the simulations, we assumed that the standard deviation of sample means due to annual effects is identical for all stocks (i.e. $s_{AVij} = \bar{s}_{AVi'}$, $i = 1, 2, 3$ and $j = 1, 2, \ldots, 42$) because empirical data were available for only 16 of the 42 stocks considered. The simulated values of $\bar{s}_{AVi}$ (0.5, 0.6, and 0.7 for the three scale factors, $i = 1,2,3$, respectively) were based on preliminary analyses of the empirical data; our revised analysis suggests that typical values of $\bar{s}_{AVi}$ in nature (the average over 16 stocks) are 0.6, 0.4, and 0.7, for $i = 1,2,3$, respectively. However, the simulated values are well within the observed range because scale patterns vary more in some stocks than others (Fig. 3). Thus, scale pattern data collected in past years might represent some stocks adequately in future years, but they are likely to be very misleading for a significant number of stocks. Bias in stock composition estimates due to annual variation will probably be reduced for mixtures of fewer stocks where annual variation is small relative to the average differences among stocks (Kathleen Jensen, ADFG, P.O. Box3-2000, Juneau, AK 99802, pers. comm). Even so, our results imply that without annual resampling, scale pattern markers will not provide reliable stock composition estimates for coast-wide mixtures involving a large number of stocks. Similarly, scale pattern markers cannot be expected to provide reliable estimates of stock composition for coast-wide mixtures during the fishing season; sockeye stocks being caught in fisheries will not yet have segregated into their natal streams so that reference samples must include scale pattern data collected in previous years or from sibling age classes.

Despite obvious problems, scale pattern and age composition analyses continue to fulfill a useful role in many stock management applications (e.g. Marshall et al. 1987). Scale samples can be collected conveniently and inexpensively from most fisheries whereas it is often difficult to collect the corresponding genetic and parasite data. Thus, the costs of scale pattern analysis are high for annual reference samples, but low for fishery samples; in contrast, the costs for genetic and parasite samples are low for reference samples, but very high for fishery samples (Wood et al. 1988). Where managers require weekly estimates of stock composition from a large number of fisheries, and where trends in stock composition may be more important than the accuracy of individual estimates, scale pattern and age composition analyses will be more cost effective than GPAS analyses. Ultimately, however, the potential capability of scale pattern analysis is limited by the number of stocks that can be distinguished reliably, or the number that can be resampled annually.

It seems unlikely that as many as 50 or 60 sockeye stocks could be sampled annually for scale pattern data, at least in the foreseeable future (about 30 stocks are sampled annually at present). Therefore, immediate application of the GPAS marker set would require that numerous small coastal sockeye stocks be omitted from the analysis and that attention be restricted to major stocks that could be sampled annually. Of course, any increase in precision resulting from the use of the full GPAS marker set or the cost-effective S marker set, for a reduced number of stocks, instead of the stable GPA set for all stocks, might be offset by reduced accuracy if in fact small unsampled stocks contributed significantly to mixed-stock fisheries. Such a priori assumptions may be worthwhile depending on the purpose of the analysis. Alternatively, if we wish to avoid making a priori judgements about contributions by minor stocks, we must rely on markers that do not exhibit annual variation, and for which reference data can be accumulated gradually with affordable annual sampling effort.

Genetic markers and the prevalence of the brain parasite, *Myxobolus neurobius* have proven to be remarkably stable from year to year (Table 3; Beacham et al. 1987; Winans and Helle 1988). This attribute, together with the simple statistical distribution of these markers, makes them ideal for complicated stock identification problems where annual resampling of reference stocks is prohibitively expensive. Unfortunately, too few genetic or stable parasite markers have been identified in sockeye salmon to achieve desired levels of stock resolution. Two other genetic loci are polymorphic in sockeye — alanine aminotransferase, *Alat*, and glyceraldehydephosphate dehydrogenase, *Gl*-2 — but the reference sampling data for these loci remains incomplete. Also, the roundworm parasite *Philonema oncorhynchi* is present in some populations and not others, and this distribution appears to be relatively stable over time (L. Margolis and C. C. Wood, Pacific Biological Station, Nanaimo, B.C., V9R 5K6, unpubl. data). The latter parasite appears especially promising for identifying Fraser River sockeye and the river-rearing Stikine stocks. However, the practicality of field sampling for *Philonema* is still being evaluated.

Using only the GPA marker set, which appears to be stable over time, the reliability of estimates of contributions by individual stocks is unacceptable in most cases (Fig. 4), even with mixture sample sizes approaching 1000 fish (Fig. 6). These estimates can be greatly improved by using the allocate-sum procedure to form stock-groupings. For example, the 15 stock-groupings denoted by Roman numerals in Fig. 2 can be identified reliably enough for most management purposes. Some of these stock-groupings are useful to managers (e.g. group I which includes all Fraser River stocks above Hell's Gate excluding Horsefly River, as well as Long Lake on the central coast). Other stock-groupings like group IV include stocks from various drainages, or from different nations, and consequently, are of limited usefulness to managers. Even so, in many cases, managers will be confident that some stocks in the group did not contribute to the fishery sample based on additional information not available to the statistical model (e.g. run timing, migration routing, fish weight, or colour).

The reliability of stock composition estimates depends very much on which stocks are present in the mixture. Consequently, it is very difficult to make general recommendations about sample sizes required to achieve acceptable estimates of mixture composition. Reliable estimates can be obtained by increasing mixture sample size for many mixture samples comprising only one stock (e.g. mixtures 1 and 4 in Fig. 6). It is disheartening to note, however, that the overall reliability of estimated proportions measured as a mean angular deviation, $\overline{\Theta}$, for mixture samples comprising several or many stocks tend to be only as good as those expected for the least identifiable stock contributing to the mixture. This problem is explored more thoroughly in a companion paper (Wood 1989).

Sophisticated techniques are currently available for coast-wide stock identification of sockeye salmon using biological markers. Unfortunately, annual variation in scale pattern data probably precludes the practical application of the full GPAS marker set required to differentiate individual sockeye stocks reliably. With continued research, however, it seems likely that additional stable markers will soon be discovered, and that the promise of existing methods will be fully realized.

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References

AMOS, M. H., R. E. ANAS, AND R. E. PEARSON. 1963. Use of a discriminant function in the morphological separation of Asian and North American races of pink salmon, *Oncorhynchus gorbuscha* (Walbaum). Int. North Pacific Fish. Comm. Bull. 11: 73–100.

Anas, R. E., and S. Murai. 1969. Use of scale characters and a discriminant function for classifying sockeye salmon (*Oncorhynchus nerka*) by continent of origin. Int. North Pacific Fish. Comm. Bull. 26: 157–192.

Bailey, R. E., and L. Margolis. 1987. Comparison of the parasite fauna of juvenile sockeye salmon (*Oncorhynchus nerka*) from southern British Columbia and Washington State lakes. Can. J. Zool. 65: 420–431.

Beacham, T. D., A. P. Gould, R. E. Withler, C. B. Murray, and L. W. Barner. 1987. Biochemical genetic survey and stock identification of chum salmon (*Oncorhynchus keta*) in British Columbia. Can. J. Fish. Aquat. Sci. 44: 1702–1713.

Beacham, T. D., R. E. Withler, and A. P. Gould. 1985a. Biochemical genetic stock identification of chum salmon (*Oncorhynchus keta*) in southern British Columbia. Can. J. Fish. Aquat. Sci. 42: 437–448.

1985b. Biochemical genetic stock identification of pink salmon (*Oncorhynchus gorbuscha*) in southern British Columbia and Puget Sound. Can. J. Fish. Aquat. Sci. 42: 1474–1483.

Bilton, H. T., and D. W. Jenkinson. 1968. Comparison of otolith and scale methods for aging sockeye (*Oncorhynchus nerka*) and chum (*O. keta*) salmon. J. Fish. Res. Board Can. 25: 1067–1069.

Bilton, H. T., and H. B. Messinger. 1975. Identification of major British Columbia and Alaska runs of age 1.2 and 1.3 sockeye from their scale characters. Int. North Pacific Fish. Comm. Bull. 32: 109–129.

Blouw, D. M., S. D. Saxon, and E. M. P. Chadwick. 1988. Temporal variation of meristic traits within an Atlantic Salmon (*Salmo salar*) stock, and implications for stock identification. Can. J. Fish. Aquat. Sci. 45: 1330–1339.

Child, A. R. 1980. Identification of stocks of Atlantic salmon (*Salmo salar* L.) by electrophoretic analysis of serum proteins. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 176: 65–67.

Cook, R. C., and G. E. Lord. 1978. Identification of stocks of Bristol Bay sockeye salmon (*Oncorhynchus nerka*) by evaluating scale patterns with a polynomial discriminant method. Fish. Bull. 76: 415–423.

Craig, P. C. 1985. Identification of sockeye salmon (*Oncorhynchus nerka*) stocks in the Stikine River based on egg size measurements. Can. J. Fish. Aquat. Sci. 42: 1696–1701.

Davis, N. D., K. W. Myers, R. V. Walker, and C. K. Harris. 1989. The Fisheries Research Institute's high seas salmonid tagging program and methodology for scale pattern analysis. Am. Fish. Soc. Proceedings of the International and Educational Workshop on Fish Marking Techniques, 27 June–1 July, 1988, Seattle, WA. (In press)

Fournier, D. A., T. D. Beacham, B. E. Riddell, and C. A. Busack. 1984. Estimating stock composition in mixed-stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400–408.

Fukuhara, F. M., S. Murai, J. J. LaLanne, and A. Sribhibhadh. 1962. Continental origin of red salmon as determined from morphological characters. Int. North Pacific Fish. Comm. Bull. 8: 15–109.

Henry, K. 1961. Racial identification of Fraser River sockeye salmon (*Oncorhynchus nerka* Walbaum) by means of scales and its application to salmon management. Int. North Pacific Fish. Comm. Bull. 12.

Lear, W. H., and E. J. Sandeman. 1980. Use of scale characters and discriminant functions for identifying continental origin of Atlantic salmon. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 176: 68–75.

MacCrimmon, H. R., and R. R. Claytor. 1985. Meristic and morphometric identity of Baltic stocks of Atlantic salmon (*Salmo salar*). Can. J. Zool. 63: 2032–2037.

1986. Possible use of taxonomic characters to identify Newfoundland and Scottish stocks of Atlantic salmon, *Salmo salar* L. Aquacult. Fish. Manage. 17: 1–17.

Margolis, L. 1963. Parasites as indicators of the geographic origin of sockeye salmon, *Oncorhynchus nerka* (Walbaum) occurring in the North Pacific Ocean and adjacent seas. Int. North Pacific Fish. Comm. Bull. 11: 101–156.

Marshall, S., F. Bergander, and S. Sharr. 1982. Origins of sockeye salmon (*Oncorhynchus nerka*) in the Lynn Canal drift gillnet fishery of 1981 based on scale pattern analysis. Alaska Department Fish Game, Tech. Data Rep. No. 75.

Marshall, S., D. Bernard, R. Conrad, B. Cross, D. McBride, A. McGregor, S. McPherson, G. Oliver, S. Sharr, and B. Van Allen. 1987. Application of scale patterns analysis to the management of Alaska's sockeye salmon (*Oncorhynchus nerka*) fisheries, p. 307–326. *In* H. D. Smith, L. Margolis, and C. C. Wood. [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future mangement. Can. Spec. Publ. Fish. Aquat. Sci. 96.

Marshall, S. L., G. T. Oliver, D. R. Bernard, and S. A. McPherson. 1984. Accuracy of scale pattern analysis in separating major stocks of sockeye salmon (*Oncorhynchus nerka* from Southeastern Alaska and northern British Columbia. Alaska Dept. of Fish. Game, Info. Leaf. No. 230. 28 p.

Messinger, H. B., and H. T. Bilton. 1974. Factor analysis in discriminating the racial origin of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Board Can. 31: 1–10.

Millar, R. B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 44: 583–590.

Milner, G. B., D. J. Teel, and F. M. Utter. 1983. Genetic stock identification study. National Oceanic and Atmospheric Administration, Northwest and Alaska Fisheries Center, Seattle WA 98112.

Milner, G. B., D. J. Teel, F. M. Utter, and C. L. Burley. 1981. Columbia River stock identification study: validation of genetic method. Annual Report of Research, National Oceanic and Atmospheric Administration, Northwest and Alaska Fisheries Center, Seattle WA 98112.

Moles, A., P. Rounds, and C. Kondzela. 1989. Use of the brain parasite *Myxobolus neurobius* in separating mixed stocks of sockeye salmon. Am. Fish. Soc. Sympos. Series (In press)

Okazaki, T. 1985. Distribution and migration of *Salmo gairdneri* and *Salmo mykiss* in the North Pacific based on allelic variation of enzymes. Jpn. J. Ichthyol. 32: 203–215.

Payne, R. H. 1980. The use of serum transferrin polymorphism to determine the stock composition of Atlantic salmon in the West Greenland fishery. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 176: 60–64.

Pearson, R. E. 1964. Use of a discriminant function to classify North American and Asian pink salmon, *Oncorhynchus gorbuscha* (Walbaum) collected in 1959. Int. North Pacific Fish. Comm. Bull. 14: 67–90.

Pella, J. J., and G. B. Milner. 1987. Use of genetic marks in stock composition analysis, p. 247–276 *In* N. Ryman and F. W. Utter. [ed.] Population genetics and fisheries management. University of Washington Press, Seattle, WA.

Pippy, J. H. C. 1980. The value of parasites as biological tags in Atlantic salmon at West Greenland. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 176: 76–81.

Quinn, T. P., C. C. Wood, L. Margolis, B. E. Riddell, and K. D. Hyatt. 1987. Homing in wild sockeye salmon (*Oncorhynchus nerka*) populations as inferred from differences in parasite prevalance and allozyme allele frequencies. Can. J. Fish. Aquat. Sci. 44: 1963–1971.

Reddin, D. G., and R. K. Misra. 1985. Hotelling's T^2 to identify the origin of Atlantic salmon (*Salmo salar*) in a mixed-stock fishery. Can. J. Fish. Aquat. Sci. 42: 250–255.

Snedecor, G. W., and W. G. Cochran. 1980. Statistical methods. 7th ed. Iowa State University Press, Ames, IO.

Steer, G. J., N. B. F. Cousens, H. Stiff, and K. D. Hyatt. 1986. An analysis of gear selectivity and sources of bias in estimates of stock composition of the 1980–1984 Barkley Sound sockeye salmon (*Oncorhynchus nerka*) catch. Can. Tech. Rep. Fish. Aquat. Sci. No. 1445: 77 p.

Utter, F., P. Aebersold, J. Helle, and G. Winans. 1984. Genetic characterization of populations in the southeastern range of sockeye salmon, p. 17–32. *In* J. M. Walton and D. B. Houston. [ed.] Proc. Olympic Wild Fish. Confer., March 23–25, 1983, Peninsula College, Port Angeles, WA.

Winans, G. A. 1984. Multivariate morphometric variability in Pacific salmon: technical demonstration. Can. J. Fish. Aquat. Sci. 41: 1150–1159.

Winans, G. A., and J. H. Helle. 1989. Electrophoretic variation in sockeye salmon (*Oncorhynchus nerka*) in southeast Alaska, Fish. Bull. (In press)

Wood, C. C., S. McKinnell, T. J. Mulligan, and D. A. Fournier. 1987a. Stock identification with the maximum-likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44: 866–881.

Wood, C. C., G. T. Oliver, and D. T. Rutherford. 1988. Comparison of several biological markers used for stock identification of sockeye salmon (*Oncorhynchus nerka*) in northern British Columbia and Southeast Alaska. Can. Tech. Rep. Fish. Aquat. Sci. No. 1624: 44 p.

Wood, C. C., B. E. Riddell, and D. T. Rutherford. 1987b. Alternative juvenile life histories of sockeye salmon (*Oncorhynchus nerka*) and their contribution to production in the Stikine River, northern British Columbia, p. 12–24. *In* H. D. Smith, L. Margolis, and C. C. Wood. [ed.] Sockeye salmon (*Oncorhynchus nerka*) population biology and future management. Can. Spec. Publ. Fish. Aquat. Sci. 96.

Wood, C. C., B. E. Riddell, D. T. Rutherford, and K. L. Rutherford. 1987c. Variation in biological characters among sockeye salmon populations of the Stikine River with potential application for stock identification in mixed-stock fisheries. Can. Tech. Rep. Fish. Aquat. Sci. No. 1535: 61 p.

Wood, C. C. 1989. The utility of similarity dendrograms in stock composition analysis. Can. J. Fish. Aquat. Sci. 46: 000–000.

Utility of Similarity Dendrograms in Stock Composition Analysis

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Similarity dendrograms are useful for revealing clustering patterns among reference stocks used in stock composition analysis. Simulations with simple mixtures of known composition indicate that clustering patterns based on genetic differences among reference stocks have a greater influence on the reliability of maximum likelihood estimates of mixture composition than either the number of reference stocks in the analysis or the size of the mixture samples. Estimated proportions for reference stocks that form tight ("problem") clusters in a similarity dendrogram tend to be biased and this bias increases as the overall proportion contributed by the problem-cluster stocks increases. Accuracy and precision can be improved dramatically by summing estimated proportions within problem clusters to estimate their overall contribution to the mixture. Adding reference stocks not present in the mixture has little effect on the reliability of stock composition estimates unless the additional stock forms a problem cluster in the similarity dendrogram. Four different measures of the degree of difference between reference stocks were evaluated for their ability to predict clustering sequences for improving estimates of mixture contributions by problem-cluster stocks.

Les dendrogrammes de similarité sont utiles pour mettre en évidence les types de groupement au sein de stocks de référence qui sont pris pour une analyse de composition des stocks. Des simulations au moyen de mélanges simples de composition connue indiquent que les genres de groupement qui s'appuient sur des différences génétiques dans les stocks de référence ont davantage d'influence sur la fiabilité des évaluation d'une probabilité maximale de composition d'un mélange que le nombre de stocks de référence servant à l'analyse ou que l'importance des échantillons des mélanges. Les proportions évaluées de stocks de référence qui forment des groupements serrés (« problèmes ») dans un dendrogramme de similarité tendent à être entachées d'une erreur systématique qui s'accroît à mesure que s'accroît l'importance des stocks composant les groupements problèmes. On peut améliorer l'exactitude et la précision d'une manière très prononcée en faisant la somme des proportions évaluées à l'intérieur des groupements problèmes, ce qui permet d'évaluer leur contribution d'ensemble au mélange. Le fait d'ajouter des stocks de référence qui ne sont pas présents dans le mélange a peu d'effet sur la fiabilité des évaluations de composition des stocks à moins que les stocks additionnels ne forment un groupement problème dans le dendrogramme de similarité. Quatre mesures différentes du degré d'écart entre des stocks de référence ont été évaluées en terme de leur capacité à laisser prévoir les séquences de groupement en vue d'améliorer les évaluations des contributions au mélange par les stocks donnant des groupements problèmes.

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The reliability of stock composition estimates obtained with the maximum likelihood mixture model (e.g. Milner et al. 1981, 1983; Fournier et al. 1984; Millar 1987) depends on the size of samples characterizing the mixture and the reference stocks, and more importantly, on differences among reference stocks with respect to the discriminating markers being used (Beacham et al. 1985; Pella and Milner 1987; Wood et al. 1987). The degree of difference ("distance") between two reference stocks is a consequence of variation in phenotype frequencies for individual markers, the number of independent markers available, and the way in which different markers act together to "key out" particular stocks. Before undertaking stock composition analyses, analysts must decide what markers to examine and what sample sizes to collect. Pair-wise distances among reference stocks can be represented graphically in similarity dendrograms in which similar stocks form clusters connected by short branches and dissimilar stocks occur in separate clusters. These diagrams can provide quick and helpful predictions about the relative reliability of estimates of mixture contributions for stocks of special interest.

First, clustering patterns in similarity dendrograms reveal which stocks are poorly differentiated ("problem clusters"), and consequently, which estimates of mixture contributions will be both biased and imprecise. By inspecting similarity dendrograms derived from various combinations of markers, investigators can select markers that maximize differences between stocks of special importance.

Second, the accuracy and precision of stock composition estimates can be improved dramatically by summing estimates within problem clusters revealed in a similarity dendrogram (the "allocate-sum" procedure of Wood et al. 1987). In contrast, little improvement in accuracy or precision can be expected by summing estimates (or pooling reference data prior to analysis) within groupings based on arbitrary schemes unrelated to similarity (e.g. geographic proximity, national boundaries).

Finally, it may be possible to recommend mixture sample sizes by identifying quantitatively the level at which stocks of special interest cluster together in a similarity dendrogram. The utility of similarity dendrograms for this purpose will depend on the extent to which other factors besides stock similarity and sample size affect the reliability of stock composition estimates.

In general, the reliability of stock composition estimates has been found to depend on the number of reference stocks considered in the analysis (Smouse et al. 1982; Wood et al. 1987). Further analysis by the author has shown that Wood et al.'s (1987) result was a special case resulting from their experimental design. In this paper, I show that under more general conditions, the reliability of maximum likelihood estimates depends primarily on clustering patterns among reference stocks revealed in similarity dendrograms and on the actual stock composition of the mixture, not on the number of reference stocks as first supposed.

Many indices have been proposed to measure distance between sets of sample data (e.g. Cavalli-Sforza and Edwards 1967; Nei 1972; 1978; Rogers 1972; Farris 1981). In this paper, I evaluate four rather different types of distance measure for their ability to highlight problem clusters in stock composition analysis with the maximum likelihood model. The first measure is Nei's (1972) genetic distance as modified by Wood et al. (1987) to accumulate rather than average, observed phenotypic differences between stocks over several genetic loci or other biological markers. The second and third measures, a log-likelihood ratio statistic, and Kullback's divergence (Kullback 1959) are designed to measure the difficulty in discriminating between a pair of hypotheses. Both indicate the likelihood that each of two random samples collected from two different stocks could have been drawn from a single stock. The fourth measure is the correlation coefficient observed between estimates of mixing proportions of the reference stocks obtained from maximum likelihood analysis of a test mixture sample. This measure is derived from a procedure suggested by Millar (1987).

I rank similarity dendrograms constructed from the four different measures according to how quickly stock composition estimates, summed within problem clusters, converge to the correct value as additional stocks are clustered in the sequence specified by each dendrogram. I also compare the performance of the clustering sequence derived from each similarity dendrogram with that of a random clustering sequence.

Methods

Experiments with Simulated Reference Stocks

The first series of simulations were designed to examine the relative importance of (1) clustering patterns among reference stocks in a similarity dendrogram, (2) mixture composition, (3) number of reference stocks used, and (4) mixture sample size, in determining the reliability of stock composition estimates from maximum likelihood analysis using genetic data.

Simulated stocks

Following Wood et al. (1987), five simulated stocks (A–E) were assigned characteristic allele frequencies at each of three loci (Table 1). All loci for a particular stock had identical allele frequencies but each locus segregated independently. Each locus had two alleles determining three genotypes in Hardy-Weinberg equilibrium. Thus, the expected (multilocus) genotype frequency distribution for a particular stock was completely specified by the frequency of one allele (denoted *a*). Distance between the simulated stocks was measured by the difference (P) in the frequency of the *a* allele between two stocks (denoted i and j). This is the P_{ij} measure recommended by Wood et al. (1987) for loci with only two alleles.

TABLE 1. Definition of simulated "stocks."

Simulated stock	Probability of *a* allele	Genotype frequencies at three independent loci		
		aa	ab	bb
A	0.1	0.01	0.18	0.81
B	0.4	0.16	0.48	0.36
C	0.5	0.25	0.50	0.25
D	0.6	0.36	0.48	0.16
E	0.9	0.81	0.18	0.01

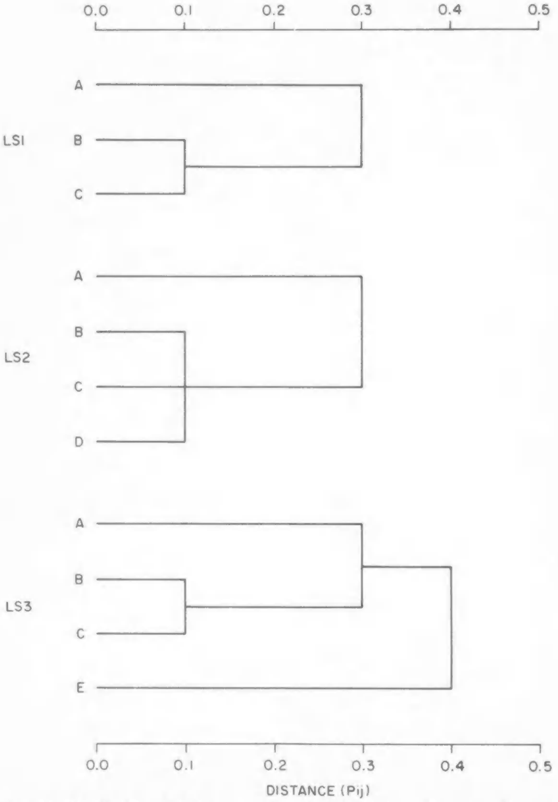

FIG. 1. Similarity dendrograms for the three learning sample sets (LS1–LS3) comprising simulated stocks A–E. The distance measure P_{ij} is the difference in the frequency of the *a* allele between any two stocks. Stocks B, C, and D are referred to as the "problem cluster" because of their similarity.

Learning samples

Three sets of the simulated stocks were used for reference ("learning samples") in stock composition analysis. The first set (LS1) comprised three stocks whereas the other sets (LS2 and LS3) comprised four stocks. These sets were selected deliberately to represent different patterns of similarity among reference stocks as revealed by the dendrograms in Fig. 1. Stocks B, C, and D are referred to collectively as the "problem cluster" because of their similarity to each other.

Mixtures

Ten mixtures of stocks A, B, and C were generated as described in Table 2 ("mixture types" No. 1–10). The overall

TABLE 2. Composition of mixtures of simulated stocks.

Mixture number	Mixture configuration	Proportion contributed by stock: A	B	C	Problem cluster total (B+C)
1	A	1.00	0.00	0.00	0.00
2	AB	0.66	0.34	0.00	0.34
3	AC	0.66	0.00	0.34	
4	ABC	0.66	0.17	0.17	
5	AB	0.34	0.66	0.00	0.66
6	AC	0.34	0.00	0.66	
7	ABC	0.34	0.33	0.33	
8	B	0.00	1.00	0.00	1.00
9	C	0.00	0.00	1.00	
10	BC	0.00	0.50	0.50	

proportion contributed by the problem cluster stocks (B and C) was 0 for mixture type 1, 0.34 for mixture types 2–4, 0.66 for mixture types 5–7, and 1.00 for mixture types 8–10. In addition, the "configuration" of mixtures within each of these four categories varied such that the problem cluster component comprised either entirely stock B (mixture configuration AB), entirely stock C (configuration AC), or equal proportions of B and C (configuration ABC).

Stock composition analysis

In the first analysis, I examined the effect of mixture configuration and proportion contributed by the problem cluster. The composition of all 10 mixture types was estimated using the maximum likelihood method of Fournier et al. (1984) and conditional probabilities calculated from the first learning sample set (LS1). Each mixture sample comprised 300 fish.

Next, I investigated the effect of number and similarity of reference stocks in the learning samples. The composition of mixture types 1, 3, 6, and 9 (all of which have configuration A, AC, or C) was estimated using all three sets of learning samples, LS1, LS2, and LS3. Again, each mixture sample comprised 300 fish.

Finally, I examined the effect of mixture sample size and learning sample set. The composition of mixture type 6 (34% A, 66% C) was estimated using all three learning sample sets in turn, for mixture sample sizes of 10, 50, 100, and 300 fish.

Monte Carlo resampling procedures were used to estimate the precision of stock composition estimates as described by Wood et al. (1987, 1989). One hundred mixture samples of each type were generated by randomly selecting the required number of "fish" (i.e. random combinations of genotypes at three loci) from each reference stock according to the probability distributions for that stock. This procedure simulates the error expected in determining genotype frequencies for a specified mixture sample size; note that the actual stock composition of the mixtures did not vary during resampling. At the same time, independent learning samples of 100 fish were drawn randomly according to the specified genotype frequency distributions to simulate error introduced while sampling the reference stocks. Genotype frequencies in the learning samples were calculated from the binomial distribution specified by the observed allele frequencies (assuming Hardy-Weinberg equilibrium within stocks). The reliability of stock composition estimates was measured by the mean angular deviation ($\bar{\theta}$) in radians of the estimated vector of stock proportions ($\hat{p}_1, \hat{p}_2, \ldots, \hat{p}_n$) from the true vector (Wood et al. 1987, 1989). This index reflects both the precision and accuracy of estimates for all stocks.

Comparison of Distance Measures

The second series of simulations was designed to evaluate three different distance measures for their ability to distinguish problem clusters in stock identification problems involving the 51 sockeye salmon (*Oncorhynchus nerka*) populations discussed by Wood et al. (1989).

Distance calculations

The first measure was Nei's (1972) genetic distance (D_{ij}) as modified by Wood et al. (1987) to accumulate differences rather than average differences in phenotype frequencies over multiple loci or other markers. The various markers are assumed to be independent of one another. This distance is referred to as the "modified Nei" distance denoted ϕ_{ij}

$$(1)\quad \phi_{ij} = \sqrt{\sum_k D_{ijk}{}^2}$$

where

$$D_{ijk} = -\log_e \left(\sum_r q_{ikr} q_{jkr} \Big/ \sqrt{\sum_r q_{ikr}{}^2 \sum_r q_{jkr}{}^2} \right)$$

for marker k and q_{ikr} and q_{jkr} are the relative frequencies of phenotype r at marker k in stocks i and j, respectively.

The second measure is a "log-likelihood ratio" distance (LR_{ij}) suggested by J. Schnute (Pacific Biological Station, Nanaimo, B.C. V9R 5K6, pers. comm.) which considers both phenotype frequencies and the size of samples from stocks.

$$(2)\quad LR_{ij} = -2 \sum_r^R \{f_{ir}\log_e(f_{ir}/n_i) + f_{jr}\log_e(f_{jr}/n_j) \\ - (f_{ir}+f_{jr})\log_e[(f_{ir}+f_{jr})/(n_i+n_j)]\}$$

where f_{ir} is the number of fish with phenotype r in stock i and n_i is the sample size for stock i. Note that R is the total number of possible phenotypes over all M markers (see Appendix for derivation). This statistic is related to Kullback's mean information statistic (I) for discrimination in favour of the hypothesis of separate stocks (H_1) against the hypothesis of a single stock (H_0) (Kullback 1959). In fact, $LR_{ij} = -2I(H_0, H_1)$.

The third measure, Kullback's divergence (J) is another measure of the information for discriminating between the two hypotheses H_0 and H_1 (Kullback 1959). In this application, it is defined as the sum of the mean information statistics computed for each of the two stocks separately

$$(3)\quad J(H_0, H_1) = \left(\frac{n_i n_j}{n_i + n_j}\right) \sum_k \left(\frac{f_{ik}}{n_i} - \frac{f_{jk}}{n_j}\right) \log_e \left(\frac{n_j f_{ik}}{n_i f_{jk}}\right)$$

The fourth measure is derived from the correlation coefficient between bootstrapped estimates for two stocks such that stocks whose estimated mixing proportions are most negatively correlated are judged to be most similar (Millar 1987). One problem with this measure is that estimated proportions must sum to one; thus, negative correlations are always expected between stocks contributing the largest number of fish to a mixture sample. For example, estimated proportions for a two-stock mixture problem will be perfectly, inversely correlated, even if the stocks are quite dissimilar. To minimize this problem, correlation coefficients between estimated proportions were computed for mixture samples comprising equal proportions of all reference stocks being considered. (This procedure was suggested by D. A. Fournier, Box 265, Nanaimo, B.C., pers. comm.). Therefore, the "correlation" measure is defined as $C_{ij}=r_{ij}+1$ where r_{ij} is the observed correlation coefficient between bootstrapped maximum likelihood estimates for stocks i and j for mixture samples comprising equal proportions of stocks i and j. Thus, $0 \leq C_{ij} \leq 2$ for all i and j.

Similarity dendrograms and clustering sequence

A matrix of pair-wise distances between stocks was computed for each of the four distance measures using the genetic, brain parasite, and freshwater age (GPA) marker set for 51 sockeye salmon (*Oncorhynchus nerka*) stocks described by Wood et al. (1989). Corresponding similarity dendrograms were constructed using the single-linkage criterion (Pielou 1977). The number of clusters defined by the dendrogram was determined over a range of distance values; critical distance values were selected for each dendrogram such that the number of clusters diminished from 51 (no clustering, zero distance) to 40, 30, 20, 10, 5, and 1 (maximum distance) as more and more dissimilar stocks or stock-groupings were clustered together. The order in which stocks are grouped together to form these clusters is referred to as the "clustering sequence" associated with a particular dendrogram. A "random" clustering sequence was also defined using a fifth dendrogram constructed by randomly reassigning among the various stock pairs, the modified Nei distances used to construct the first dendrogram.

Comparison of clustering sequences

To evaluate the performance of each distance measure, I summed estimated mixing proportions of stocks for simulated mixtures of known composition according to the clustering sequence of the distance measure (the allocate-sum procedure described by Wood et al. 1987, 1989). For simplicity, the simulated mixtures comprised only a single stock. Therefore, the estimated mixing proportion for the contributing stock or group containing that stock always improved along the clustering sequence. Clustering sequences that provided the earliest improvement in estimated mixing proportions were considered to be most useful. For each clustering sequence, mean errors were summed across the 40, 30, 20, 10, and 5 stock-grouping levels. Clustering sequences resulting in a large summed error statistic were slower to converge to the correct solution than those resulting in a small summed error statistic.

Stock composition analysis

Stock composition was estimated for 10 new simulated mixture types, each consisting of 300 fish from a single stock (denoted No. 11–20), using the GPA marker set and 51 reference stocks of sockeye salmon described by Wood et al. (1989). Mixture types No. 11–15 were identical to mixture types 1 to 5 in Wood et al. (1989) where contributing stocks were selected to provide a range of mixture problems ranging from easy (100% Tahltan Lake) to difficult (100% McDonald Lake). Stocks contributing to the mixture types No. 16–20 were chosen randomly from the remaining 46 sockeye stocks (Table 3). Monte Carlo resampling techniques were used to generate 100 estimates for each mixture type following the same procedures as Wood et al. (1989).

To estimate the correlation distance, 100 estimates of mixing proportions were obtained using the GPA marker set and identical procedures, for a mixture consisting of 306 fish, six fish from each of the 51 reference stocks.

Results

Effect of Mixture Composition

In the analyses with simulated stocks, estimates of mixture composition proved to be very sensitive to the proportion of the mixture that was contributed by problem cluster stocks, B and C (Fig. 2). Composition estimates were biased most seriously when problem cluster stocks made up more than half of the mixture sample. However, estimates of composition were relatively unaffected by the configuration of the mixture; that is, it made little difference which of the problem cluster stocks actually contributed to the mixture sample. Other things being equal, estimates were slightly better for mixtures composed of stocks A and C than for those composed of A and B. This is attributed to the fact that the distance between stocks A and B, ($P_{AB}=0.3$) is slightly less than that between stocks A and C ($P_{AC}=0.4$). Even so, these distances are large compared with the distance between problem cluster stocks ($P_{BC}=P_{CD}=0.1$).

Effect of Learning Sample Set

Estimates of mixture composition were also very sensitive to the particular learning sample set used in the analysis, especially where problem cluster stocks predominated in the mixture sample (Fig. 2). Mixture samples comprising only stocks A, AC, or C were reanalyzed with different learning sample sets (LS2 and LS3) which included an additional stock (D and E, respectively) not present in the mixture samples. The reliability of the resulting estimates of composition decreased markedly when the additional reference stock fell within the problem cluster (stock D in LS2) but decreased only slightly if the additional stock fell outside the problem cluster (stock E in LS3) (Fig. 2). Thus, the reliability of stock composition estimates is more influenced by the clustering pattern among reference stocks than by the number of reference stocks in the learning samples.

Effect of Mixture Sample Size and Clustering Procedures

Mixture composition and clustering pattern among reference stocks in the learning samples were the predominant factors influencing the reliability of stock composition estimates for all mixture sample sizes examined (Fig. 3). For the mixture comprising 34% stock A and 66% stock C, estimates of composition improved considerably (by 41–44%) as mixture sample sizes increased from 10 to 100 fish using learning sample sets LS1 and LS3. In contrast, very little improvement (~10%) was observed for the same analysis using learning sample set LS2.

Summing estimates within the problem cluster greatly improved the reliability of composition estimates for these simulated stocks at all mixture sample sizes (Fig. 3). This is because estimated contributions by stocks outside the problem cluster (A and E) were usually very reliable. Thus, despite the fact that individual contributions by the problem cluster stocks (B, C, and D) were poorly determined, the collective contribution by the problem cluster was usually determined very accurately.

Comparison of Distance Measures

Five clustering sequences were determined from patterns of similarity among 51 sockeye stocks using different measures of distance between stock pairs (see Methods). Estimates of composition were summed within stock-groupings according to these sequences for 10 different mixture types, each comprising

TABLE 3. Comparison of performance of five clustering sequences for 10 mixture problems with 51 sockeye salmon reference stocks. Values are mean error statistics summed over 40, 30, 20, 10, and five stock-grouping levels. Low values indicate rapid improvement of estimates summed within groupings. Numbers in parentheses are rank scores.

		Distance measure used to derive clustering sequence				
Mixture composition	Stock number[a]	Modified Nei	Likelihood ratio	Kullback divergence	Correlation	Random
No. (11) 100% Stikine[b]	12	1.03 (2)	1.02 (1)	1.11 (4)	1.57 (5)	1.05 (3)
No. (12) 100% Babine[b]	15	0.07 (1)	0.22 (2.5)	0.22 (2.5)	0.35 (4.5)	0.35 (4.5)
No. (13) 100% Shuswap[b]	3	0.21 (3)	0.18 (2)	0.24 (4)	0.12 (1)	0.29 (5)
No. (14) 100% Tahltan[b]	24	0.20 (4)	0.20 (4)	0.20 (4)	0.07 (1)	0.12 (2)
No. (15) 100% McDonald[b]	40	1.50 (2)	1.43 (1)	1.68 (3)	2.92 (5)	2.12 (4)
No. (16) 100% Kegan	44	0.65 (3)	0.69 (4)	0.72 (5)	0.43 (1)	0.44 (2)
No. (17) 100% Canoona	25	0.09 (3)	0.11 (4)	0.14 (5)	0.06 (2)	0.05 (1)
No. (18) 100% Long	4	0.31 (1)	0.70 (2)	0.73 (3)	1.00 (5)	0.95 (4)
No. (19) 100% Bowser	6	0.45 (4)	0.35 (2.5)	0.35 (2.5)	0.17 (1)	0.53 (5)
No. (20) 100% Owikeno	32	0.16 (1)	0.20 (2)	0.47 (4)	0.41 (3)	1.02 (5)
Overall mean		0.47	0.51	0.59	0.71	0.69
Sum of rank scores		(24.0)	(25.0)	(37.0)	(28.5)	(35.5)

[a] Corresponds to number in figure 1, Wood et al. (1989).

[b] Mixtures analyzed by Wood et al. (1989).

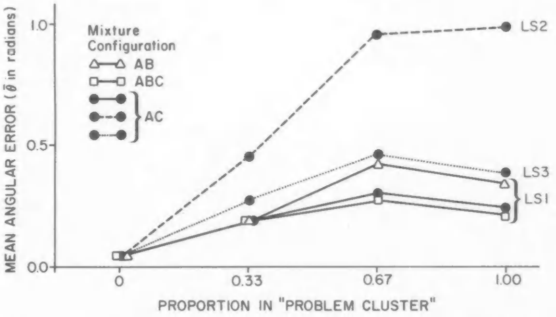

FIG. 2. Effect of mixture composition and learning sample set (LS1–LS3) on the reliability of stock composition estimates measured by the mean angular error statistic. The "proportion in problem cluster" refers to the overall contribution by problem–cluster stocks B or C. Mixture configuration is defined in Table 2, and learning sample set, in Fig. 1. All simulated mixtures consisted of 300 fish.

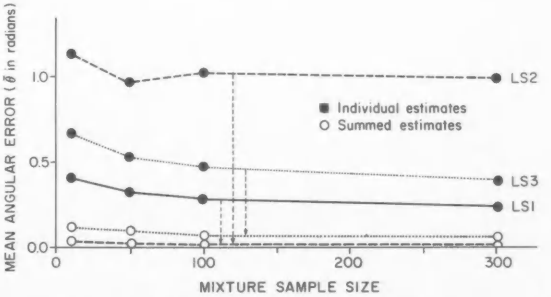

FIG. 3. Effect of learning sample set over a range of mixture sample sizes. All mixtures consisted of 34% stock A and 64% stock C (configuration AC in Fig. 2). Open symbols indicate the corresponding error rate when estimated proportions for problem-cluster stocks B–D are summed.

only one of 51 possible stocks. The reliability of the summed estimates improved for all clustering sequences and all mixtures, but the positions in the clustering sequences at which estimates improved varied widely among distance measures (Fig. 4). The mixture types in Fig. 4 are those resulting in the four worst estimates of contribution by the individual stocks in question; consequently, these examples demonstrate the greatest potential for improving estimates by summing within stock-groupings. The modified Nei and log-likelihood ratio clustering sequences resulted in the most rapid convergence to the correct result, or similarly, to an acceptable result. The Kullback divergence sequence performed only slightly less well than the likelihood ratio sequence; it was omitted from Fig. 4 for the sake of clarity. In contrast, the correlation clustering sequence did not converge significantly faster than the random clustering sequence in these examples.

The results illustrated in Fig. 4 are typical of the results for the other six mixture types. The rate of convergence was measured by summing the mean estimated error (difference between the mean estimated proportion and the correct result of 1.00) across five clustering levels (see Methods). Thus, a large summed error statistic indicates slow convergence to the correct result. When distance measures were ranked according to the average summed error statistic for all 10 mixture types, the modified Nei distance measure performed best (mean = 0.47), the log-likelihood ratio measure came a close second (mean = 0.51), and the Kullback divergence measure, third (mean = 0.59) (Table 3). The clustering sequence based on the correlation distance performed no better than the random clustering sequence error statistic (mean = 0.71 compared with 0.69, respectively). However, when the summed error statistics were assigned rank scores (from 1 to 5 for each mixture type), and the scores were summed over all mixtures, the correlation clustering sequence performed considerably better than the random sequence (summed rank score = 28.5 compared with 35.5). Again, the modified Nei sequence performed best (24), followed closely by the log-likelihood ratio sequence (25). Overall, the Kullback divergence sequence performed poorly

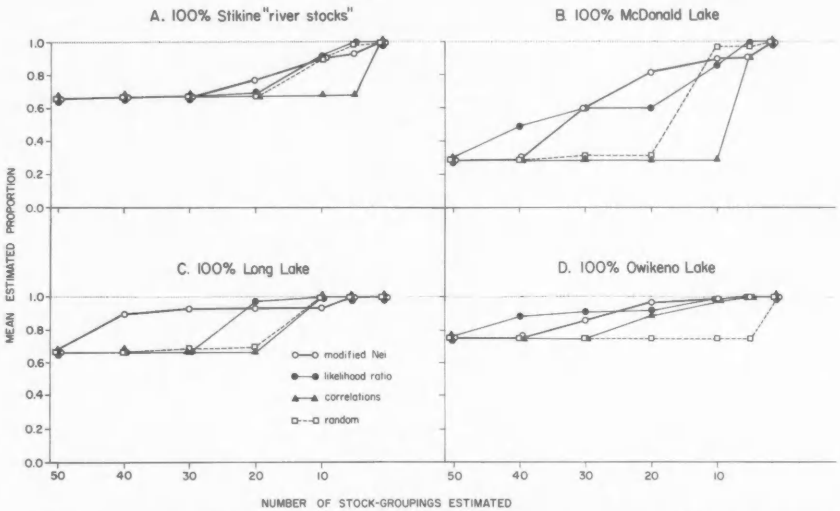

FIG. 4. Comparison of four clustering sequences with respect to the rate at which mean estimated proportions summed within clusters converge to the correct solution as the clusters become larger (and the number of stock-groupings become fewer). At the 51 stock-groupings level, all estimated proportions are for individual stocks whereas at the one stock-grouping level, all estimated proportions have been summed within a single large cluster. The correct proportion is 1.00 in each case. Clustering sequences resulting in a rapid convergence to the correct solution are most useful.

(summed rank score = 37.0) because it never ranked first or second for any particular mixture.

The clustering sequence based on the modified Nei distance tended to perform best at low levels of clustering (40 or 30 stock-groupings) whereas that based on the log-likelihood ratio distance usually resulted in more complete convergence of estimates to the correct values at high levels of clustering (20, 10, or 5 stock-groupings). For example, when performance was evaluated by assigning rank scores to mean errors in estimated proportion at either the 40 or the 30 stock-grouping level (rather than by summing across all levels), the modified Nei distance performed slightly better (summed rank scores = 27.5 and 25.0 for the 40 and 30 stock levels, respectively) than the likelihood distance measure (summed rank scores = 29.0 and 28.5).

Discussion

The simple mixture problems with simulated data analyzed here and elsewhere (Wood et al. 1987), indicate that the degree of difference between reference stocks has a greater influence on the reliability of maximum likelihood estimates of stock composition than either the number of reference stocks in the learning sample set or the number of fish in the mixture sample. The present analysis suggests that clustering patterns among reference stocks revealed in similarity dendrograms are extremely useful for predicting the relative reliability of stock composition estimates. Thus, discriminating markers can be selected for their capacity to produce clustering patterns that meet management objectives. Sometimes reference stocks that link together closely in dendrograms should be considered as units (i.e. problem clusters). Often a problem cluster's contribution to a mixture sample is estimated accurately even when contributions by its component stocks are not.

The reliability of stock composition estimates typically deteriorates as more reference stocks are included in the learning sample set (Smouse et al. 1982; Wood et al. 1987). However, most of this deterioration probably occurs because the chance of observing problem clusters increases as additional reference stocks are considered. In fact, maximum likelihood estimates of contributions by stocks that are not grouped in problem clusters appear to be relatively unaffected by the number of reference stocks used in the analysis (Fig. 2).

Wood et al. (1987) concluded that the number of fish per stock contributing to the mixture sample, not the number of reference stocks, affected the reliability of estimates of composition. However, they examined a special case where all reference stocks were separated by a constant increment on a P_{ij} distance scale from 0 to 1, and where all stocks contributed equally to the simulated mixtures. In other words, their simulated reference stocks formed a single large problem cluster. Given these conditions, and constant mixture sample size, the reliability of stock composition estimates can be expected to decrease through chance alone as the number of reference stocks is increased (Smouse et al. 1982). These conditions are very unlikely to prevail in practical applications. In general, I would expect the clustering pattern among reference stocks to be of much greater concern than the overall number of reference stocks in the analysis.

Maximum likelihood estimates of stock proportions become unbiased as mixture and reference samples become large provided that no two reference stocks have identical phenotype frequencies (Fournier et al. 1984; Millar 1987). In principle then, stock composition can be estimated to any desired level of precision or accuracy simply by increasing mixture and reference sample sizes. Because the reliability of stock composition estimates are so strongly influenced by clustering patterns, it should be possible to predict required mixture sample sizes by identifying quantitatively the level at which stocks of particular interest cluster together in a similarity dendrogram. Simulated mixtures involving different levels of clustering among reference stocks can be calibrated as to the reliability of stock composition estimates over a range of mixture sample sizes. A similarity dendrogram could then be used to indicate which simulated mixture corresponds most closely to the problem at hand, and should allow an analyst to infer the sample size required for any desired level of precision and accuracy. Unfortunately, if estimates of contributions by problem cluster stocks are unacceptable even with rather large mixture samples (100–300 fish), mixture samples sizes probably would have to be increased by one or several orders of magnitude to gain significant improvement (Fig. 3; also Fig. 5 in Wood et al. 1989). Thus, the analyst faced with unacceptable estimates of contributions by problem cluster stocks usually must attempt to simplify the mixture problem.

Mixture problems involving problem cluster stocks can be dealt with in several ways: (1) new discriminating markers can be evaluated for their ability to "break apart" problem clusters; (2) reference stocks within a problem cluster that are considered, a priori, to be a minor constituent of the mixture can be omitted; and (3) estimates of contributions by individual problem cluster stocks can be summed to provide a reliable estimate of the overall contribution by the problem cluster. Option (1) is most desirable but often impractical. Option (2) offers the advantage of reduced computing time and sampling effort, but depends critically on whether the a priori assumptions about mixture composition are sound. A safer procedure would be to complete the analysis with all reference stocks and use option (3) to obtain a reliable estimate for the overall contribution by the problem cluster; this estimate can then be interpreted as applying only to those problem cluster stocks expected to contribute significantly to the mixture sample. The procedure of pooling data from different reference stocks to form a reduced number of composite reference stocks is not recommended (see Wood et al. 1987) except where samples from nearby stocks are not significantly different and can reasonably be treated as a single population or group of related populations.

A similarity dendrogram is a two-dimensional representation of clustering patterns in a multi-dimensional space. Therefore, a clustering sequence derived from this representation will never be entirely successful at eliminating biases in estimates of stock composition. Even so, clustering sequences based on the modified Nei and the log-likelihood ratio distances were much more successful at reducing bias in estimated proportions than the random clustering sequence. In contrast, the clustering sequence based on the correlation distance was not much more efficient at reducing bias than the random clustering sequence.

Millar (1987) suggested that stock composition estimates could be improved by summing estimates according to a unique clustering sequence determined from correlations among estimates for a particular mixture sample (rather than the general clustering sequence based on a test mixture such as described here). However, a potential problem with Millar's (1987) suggested procedure is that estimated proportions for two or more dominant stocks in any particular mixture will tend to be negatively correlated, regardless of their similarity. This is because estimates of stock proportions are constrained to sum to one; in the extreme case, the correlation between estimates of composition for any two-stock mixture problem will always be −1.00. For this reason, I modified the procedure suggested by Millar (1987), reasoning that the strongest indication of similarity among stocks should be apparent in the negative correlations among estimated stock proportions for a simulated mixture comprising equal contributions from all stocks. Unfortunately, this procedure failed to perform as well as the other distance measures, probably due to the effect of spurious, random correlations that tended to obscure the pattern of similarity among stocks.

Both the modified Nei and log-likelihood ratio distance measures performed well. Clustering sequences based on the modified Nei distance ranked only slightly better than those based on the log-likelihood ratio distance using the mean error statistic summed over the various clustering levels. In the 10 examples tested here, there was a general tendency for the modified Nei clustering sequence to produce more rapid, but less complete convergence of estimated proportions than the log-likelihood ratio clustering sequence. For practical application, it is desirable to obtain rapid improvement at low clustering levels because stock composition estimates become less useful as more stocks are grouped together. However, the log-likelihood ratio distance has a better statistical basis in hypothesis testing, and takes into account the size of the reference samples. The likelihood ratio distance would therefore seem to be the best overall choice of the measures tested here, for assessing differences between stocks and for constructing similarity dendrograms. Of course, other distance measures might perform equally well. Further improvement in determining clustering sequences is probably limited by the two-dimensional nature of similarity dendrograms, or by the method for constructing dendrograms (single versus complete linkage, etc.), rather than the distance measure used in their construction.

Stock composition analysis is only useful when the analyst recognizes the limitations of the method. Estimates of composition can always be generated and it is not always easy to determine whether these estimates are reliable (e.g. Mulligan et al. 1988). Clustering patterns revealed in similarity dendrograms provide a helpful synopsis of the problems that can be expected in undertaking particular analyses. They allow the analyst to evaluate the utility of various discriminating markers and to recognize stock-groupings that should be treated as units. In fact, clustering patterns among reference stocks appear to be the single most important factor affecting the reliability of stock composition estimates. Thus, it should be possible to use similarity dendrograms quantitatively to ascertain the relative difficulty of mixture problems involving different markers or reference stocks. This capability would greatly facilitate the analyst's task of estimating sample sizes and stock-grouping levels required to obtain acceptable solutions to a large variety of practical problems.

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References

BEACHAM, T. D., R. E. WITHLER, AND A. P. GOULD. 1985. Biochemical genetic stock identification of chum salmon (*Oncorhynchus keta*) in southern British Columbia. Can. J. Fish. Aquat. Sci. 42: 437–448.

CAVALLI-SFORZA, L. L., AND A. W. F. EDWARDS. 1967. Phylogenetic analysis: models and estimation procedures. Evolution 21: 550–570.

FARRIS, J. S. 1981. Distance data in phylogenetic analysis, p. 3–23 *In* V. A. Funk and D. R. Brooks [ed.] Advances in cladistics: Proceedings of the first meeting of the Will Hennig Society. New York Botanical Garden, Bronx, NY.

FOURNIER, D. A., T. D. BEACHAM, B. E. RIDDELL, AND C. A. BUSACK. 1984. Estimating stock composition in mixed-stock fisheries using morphometric, meristic and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41: 400–408.

KULLBACK, S. 1959. Information theory and statistics. John Wiley & Sons, New York, NY. 395 p.

MILLAR, R. B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 44: 583–590.

MILNER, G. B., D. J. TEEL, AND F. M. UTTER. 1983. Genetic stock identification study. National Oceanic and Atmospheric Administration, Northwest and Alaska Fisheries Center, Seattle WA 98112.

MILNER, G. B., D. J. TEEL, F. M. UTTER, AND C. L. BURLEY. 1981. Columbia River stock identification study: validation of genetic method. Annual Report of Research, National Oceanic and Atmospheric Administration, Northwest and Alaska Fisheries Center, Seattle WA 98112.

MULLIGAN, T. J., S. MCKINNELL, AND C. C. WOOD. 1988. Uncertainty in stock composition estimates of oceanic steelhead trout using electrophoretic characteristics: comments on a recent study. Can. J. Fish. Aquat. Sci. 45: 432–442.

NEI, M. 1972. Genetic distance between populations. Am. Nat. 106: 283–292.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

PELLA, J. J., AND G. B. MILNER. 1987. Use of genetic marks in stock composition analysis, p. 247–276 *In* N. Ryman and F. W. Utter. [ed.] Population genetics and fisheries management. University of Washington Press, Seattle, WA.

PIELOU, E. C. 1977. Mathematical ecology. John Wiley and Sons, Inc., New York, NY 385 p.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Studies in Genetics VII. Univ. Texas Publ. 7213: 145–153.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry. 2nd ed. W. H. Freeman and Co., San Francisco, CA 859 p.

SMOUSE, P. E., R. S. SPIELMAN, AND M. H. PARK. 1982. Multiple-locus allocation of individuals to groups as a function of the genetic variation within and differences among human populations. Am. Nat. 119: 445–463.

WOOD, C. C., S. MCKINNELL, T. J. MULLIGAN, AND D. A. FOURNIER. 1987. Stock identification with the maximum likelihood mixture model: sensitivity analysis and application to complex problems. Can. J. Fish. Aquat. Sci. 44: 866–881.

WOOD, C. C., D. T. RUTHERFORD, AND S. MCKINNELL. 1989. Identification of sockeye salmon (*Oncorhynchus nerka*) stocks in mixed-stock fisheries in British Columbia and Southeast Alaska using biological markers. Can. J. Fish. Aquat. Sci. 46: 000–000.

Appendix

Derivation of the Log-likelihood Ratio Distance

A random sample of n_i fish is obtained independently from each of N stocks ($i=1,\ldots,N$). Each fish is examined to determine its phenotype at each of M discriminating markers. First consider the case where $M=1$ and there are m possible phenotypes ($r=1,\ldots,m$). Let f_{ir} denote the number of fish in the sample from stock i with phenotype r. Then $\hat{q}_{ir}=f_{ir}/n_i$ is the maximum likelihood estimator of the phenotypic frequency in stock i (q_{ir}). The likelihood function for a sample from stock i is

$$L(f_{i1},\ldots f_{im}|q_{i1},\ldots,q_{im}) = [n_i!/(f_{i1}!,\ldots f_{im}!)] \prod_r q_{ir}^{f_{ir}}.$$

Under the null hypothesis that stocks i and j are identical (i.e. $q_{ir}=q_{jr}=q_r$ for all r), the likelihood function for the combined samples from stocks i and j is

$$\begin{aligned} L_0 &= L(f_{i1},\ldots f_{im}, f_{j1},\ldots f_{jm}|q_1,\ldots,q_m) \\ &= \log(n_i+n_j)!/(f_{i1}+f_{j1})!,\ldots,(f_{im}+f_{jm})!] \prod_r q_r^{(f_{ir}+f_{jr})} \end{aligned}$$

Alternatively, if $q_{ir} \neq q_{jr}$, then the appropriate likelihood function is

$$\begin{aligned} L_1 &= L(f_{i1},\ldots f_{im}|q_{i1},\ldots,q_{im})L(f_{j1},\ldots f_{jm}|q_{j1},\ldots,q_{jm}) \\ &= [n_i!/(f_{i1}!,\ldots f_{im}!)]\ [n_j!/(f_{j1}!,\ldots f_{jm}!)] \prod_r q_{ir}^{f_{ir}}\, q_{jr}^{f_{jr}}, \end{aligned}$$

since the samples are independent. The "log-likelihood ratio distance" is defined as

$$\begin{aligned} LR_{ij} = -2\log_e(L_0/L_1) = -2 \sum_r \{&f_{ir}\log_e q_{ir} + f_{jr}\log_e q_{jr} \\ &- (f_{ir}+f_{jr})\log_e q_r\}. \end{aligned}$$

Substituting f_{ir}/n_i for q_{ir} and $(f_{ir}+f_{jr})/(n_i+n_j)$ for q_r yields

$$\begin{aligned} LR_{ij} = -2 \sum_r \{&f_{ir}\log_e(f_{ir}/n_i) + f_{jr}\log_e(f_{jr}/n_j) \\ &- (f_{ir}+f_{jr})\log_e[(f_{ir}+f_{jr})/(n_i+n_j)]\}. \end{aligned}$$

Like the G-statistic (Sokal and Rohlf 1981), the distribution of LR_{ij} is approximately as χ^2 with $m-1$ degrees of freedom.

In the more general case where there are several markers ($M>1$), each fish will have M phenotypes. Providing the markers are independent, the set of possible phenotypes can simply be expanded so that $r=1,\ldots,R$ and LR_{ij} is calculated as before replacing m and R. Note, however, that when $M>1$, LR_{ij} is distributed as χ^2 with $R-M$ degrees of freedom because phenotypic frequencies must sum to one for each marker; thus, one degree of freedom is lost for each.

Trends in Fishing Mortality Rate along with Errors in Natural Mortality Rate can cause Spurious Time Trends in Fish Stock Abundances Estimated by Virtual Population Analysis (VPA)

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Many researchers have reported biases in estimates of fish abundance reconstructed by virtual population analysis (VPA). We document that VPA can produce changing levels of bias through time, thereby creating spurious time trends in recruitment and stock biomass estimates. We generated catch data from empirically based simulations of nine fish populations, estimated abundances using VPA with a deliberately mis-specified natural mortality rate, *M*, and compared the estimates to the models' "true" abundances. A period of increasing fishing mortality rate, *F*, combined with an overestimate of *M*, produced spurious decreasing time trends in estimated abundance and recruitment, even when the true time series of *F* was known. Analogously, an underestimate of *M* led to a spurious increasing time trend. Bias was increased by a higher true *M*, and (for a given total change in *F*) by a slower increase in *F*. Because field estimates of *M* are uncertain and trends in *F* are common, some apparent trends (or lack of them) in abundances reconstructed by VPA may be artifacts. Therefore, inferences about the results of past management actions and about physical or biological effects on variability in recruitment must be made cautiously when VPA estimates are used.

De nombreux chercheurs ont signalé l'existence de biais dans les estimations des effectifs de poissons réalisées par l'analyse des populations virtuelles (VPA). Nous soutenons que les biais inhérents à l'exécution de la VPA peuvent varier dans le temps, introduisant ainsi de fausses tendances temporelles dans les estimations du recrutement et de la biomasse des stocks. Nous avons produit des données sur les captures à partir de simulations empiriques de neuf populations de poissons, estimé les effectifs au moyen de la VPA fondée sur un taux de mortalité naturelle délibérément faussé (*M*) et comparé les estimations aux effectifs « véritables » calculés par les modèles. Une période d'accroissement du taux de mortalité due à la pêche (*F*), alliée à une surestimation de *M*, a engendré des tendances temporelles anormalement décroissantes dans les estimations des effectifs et du recrutement, même quand la véritable série chronologique de *F* était connue. En corollaire, la sous-estimation de *M* a produit une fausse tendance temporelle croissante. Le biais augmentait quand le véritable taux de mortalité naturelle augmentait et quand la valeur de *F* croissait plus lentement (pour un changement total donné de cette valeur). Puisque les estimations de M sur le terrain sont peu sûres et que les tendances de la valeur de *F* sont courantes, la présence — ou l'absence — de tendances apparentes dans les effectifs établis par la VPA pourrait bien être artificielle. En conséquence, quand on utilise les estimations produites par la VPA, il importe d'user de prudence pour faire des inférences relatives aux résultats d'activités de gestion passées et aux effets des conditions physiques ou biologiques sur la variabilité des taux de recrutement.

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Virtual population analysis (VPA) and related sequential population estimation techniques (Gulland 1983; Murphy 1965; Pope 1972; Rivard 1983; MacCall 1986) are widely used to reconstruct abundances of fish stocks. This reconstruction is an important component of standard procedures of many fisheries management agencies for estimating stock sizes, forecasting catches, and evaluating the success of past management schemes (Pope and Shepherd 1985; Rivard and Foy 1987).

The effects of using incorrect parameter values in VPA have been studied extensively (e.g. Pope 1972; Agger et al. 1973; Ulltang 1977; Sims 1984; Hilden 1988). It is well known from these and other papers that errors in the instantaneous natural mortality rate, M, and the instantaneous terminal fishing mortality rate, F_L, can create erroneous abundance estimates of both recruits and stock biomass, and that the effect of errors in F_L is reduced by large cumulative fishing mortality. In addition, errors in reported catches or in estimated weights and ages of fish can also lead to incorrect abundances (Pope and Gray 1983; Rivard 1983; Rivard and Foy 1987). Because of these sources of error, several authors have derived confidence intervals for abundances estimated by VPA under various types of uncertainty in inputs to VPA (e.g. Pope 1972; Prager and MacCall 1988; Sampson 1987, 1988; Kimura 1989). Under certain conditions, errors in M, F_L or both can also create spurious time trends in abundance (Rivard 1983; Hilden 1988; Sampson 1988; Bradford and Peterman 1989); i.e. even if true recruits and stock biomass were constant, such errors in the parameters of VPA would lead to a time trend in abundance estimates. Time trends in estimated abundance are especially relevant for, (1) evaluations of past management regulations based on the estimated response of the population, and (2) research on mechanisms causing temporal variation in recruitment.

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The purpose of this paper is to document a much wider range of situations than previously reported in which spurious time trends in estimated abundances can occur. Specifically, if there are increasing trends in F in any part of the time series of catch data, and if an error exists in the M used in VPA, abundances reconstructed by VPA will have a spurious time trend.

Increasing time trends in F are common in many commercial fisheries (e.g. Hoag and McNaughton 1978; Anderson and Paciorkowski 1980; Winters et al. 1985; Espino and Wosnitza-Mendo 1986; Ahrenholz et al. 1987). These trends may result from a variety of mechanisms including increases in fleet size, "fishing-up" (the removal of accumulated stock, particularly old age classes) early in the development of a fishery (e.g. Ricker 1973; Francis 1986), use of nominal or incompletely standardized effort data which contain trends in fishing power, depensatory catchability coupled with decreases in stock size (e.g. clupeoids; MacCall 1976; Ulltang 1976), and others. Results from VPA often show increasing time trends in fishing mortality rates. However, as we document below, VPA will not correctly identify or account for such trends if there is an error in one or more of the parameters input to VPA, because trends in F exaggerate the effect of those errors in a different way than documented previously. Hilden (1988) showed that increasing trends in F biased recruitment estimates obtained from VPA, but he did not quantify their effect on time trends in recruitment or total stock biomass estimates. Analysts can, in theory, delete data covering years of increasing F from a catch data set before running VPA, but in practice there may be too few years of data left after deletion to be useful.

Methods

In nature, the true abundance of a fish stock will always be uncertain. Therefore, to investigate the impact of increasing trends in F on VPA, we used a simulation model to generate synthetic (i.e. "true") abundances that were known exactly. We focused our analyses on trends of increasing F with time, because they are the most common.

Historical F trends have differed among stocks, varying from small to large ranges in absolute F and short to long periods over which F changes occurred. Therefore, to generalize our findings, we based our analysis on nine different stocks.

The general procedure was:

1. Generate a time series of simulated "true" abundance and catch data from a population model, using known parameters and a time series of increasing fishing mortality rates to simulate the trends in F (called an "F scenario").
2. Estimate annual abundances using iterative VPA (Rivard 1983) given: (a) the catches generated by the simulation of the population model, (b) the true time series of fishing mortality rates, and (c) an incorrect M^{VPA} (henceforth, parameters with a VPA superscript will refer to the parameter values input to VPA, and those without superscripts will be the true values used in the simulation to generate the catch data).
3. Compare time trends of estimated and true abundances (recruits and biomasses).

The specifics of this procedure are as follows.

Simulated Populations and F Scenarios

Simulated populations were generated using parameters (fishing and natural mortality rates, age-specific selectivities, and weights-at-age) from nine stocks (Table 1). We chose these stocks as examples of fishes with different life histories, age-specific selection coefficients, and F trends. We used the parameter values in Tables 2 and 3 as well as others drawn from the references in Table 1. Populations were simulated using the following approach.

Recruitment (number of fish in the youngest fished age) was assumed constant at 1×10^6 fish (we relax this assumption later in the Sensitivity Analysis section), and numbers of fish in older ages were calculated using

$$(1)\quad N_{i+1} = N_i\, e^{-Z_i},$$

where N is the initial number of fish of age i and $Z_i = M + F_i$. M and F_i are the instantaneous annual natural mortality rate and age-specific fishing mortality rate, respectively. We assumed that the population had been fished at the minimum F prior to the start of each F scenario (see below).

Age-specific fishing mortality rates were calculated as

$$(2)\quad F_i = s_i\, F_L,$$

where the s_i's are the age-specific selection coefficients and F_L is the fishing mortality rate for the oldest age in a given year. F_L's for each year were determined from scenarios used to simulate periods of increasing F (see below). By assuming that the time series of F_L was known exactly for VPA, we were able to document the minimum qualitative effects of errors in M^{VPA}. We relax this assumption of known F_L later.

Stock biomass in each year was calculated as

$$(3)\quad B_t = \sum_i N_i w_i,$$

where w_i are the weights at each age in kilograms.

Simulated catches were generated using the Baranov catch equation (Ricker 1975).

$$(4)\quad C_i = \frac{N_i\, F_i\,(1 - e^{-Z_i})}{Z_i},$$

where C_i is the number of fish caught at age i.

We used two different F scenarios. In the first scenario, we used increasing F trends estimated from the historical data for each stock, to explore the relative magnitude of potential bias in abundance estimates for historical situations. Each historical F pattern was approximated with a linear trend using the observed maximum and minimum F values ($F_{\max}$ and $F_{\min}$) and the actual number of years of increasing F observed for each stock (Table 3). In the second scenario, we applied a common F trend to all stocks to generalize our results by exploring how the bias in estimated abundances was affected by different life history characteristics. In both F scenarios, F_L for each year was calculated using

TABLE 1. Sources of data used to generalize effects of input errors to VPA across stocks.

Common name	Location	Species	Abbreviation	Source
Atlantic mackerel	ICNAF areas 3–6	*Scomber scombrus*	Mac	Anderson and Paciorkowski (1980)
Atlantic menhaden	East coast, US.	*Brevoortia tyrannus*	Men	Hightower and Grossman (1985) Arenholz et al. (1987)
Dover sole	Oregon	*Microstomus pacificus*	Dov	Hayman et al. (1980)
Fortune Bay herring	Fortune Bay, Nfld.	*Clupea harengus*	FBH	Winters et al. (1985)
Pacific cod	Hecate Strait, B.C.	*Gadus macrocephalus*	Cod	Fournier (1983)
Pacific halibut	Area 3	*Hippoglossus stenolepis*	Hal	Hoag and McNaughton (1978)
Pacific ocean perch	Queen Charlotte Sound, B.C.	*Sebastes alutus*	POP	Hightower and Grossman (1985) Archibald et al. (1983)
West Scotland herring	ICES area VIa	*Clupea harengus*	WSH	Saville and Bailey (1980)
Yellowfin sole	Eastern Bering Sea	*Limanda aspera*	Yel	Wakabayashi (1984)

TABLE 2. Parameter values for nine different marine fish stocks used in the analysis. Abbreviations of stock names in the top row are defined in Table 1, where literature sources are also given. *M* is the instantaneous annual natural mortality rate. Selection coefficients (i.e. s_i) of partial recruitment to the fishery are given for each fished age, scaled so that the coefficient for the last fished age class is 1.0 in all cases. Unfished ages are shown by a hyphen.

	Stock								
	Mac	Men	Dov	FBH	Cod	Hal	POP	WSH	Yel
M	0.3	0.45	0.2	0.2	0.65	0.2	0.05	0.1	0.25
Age	Selection coefficients								
0	—	0.34	—	—	—	—	—	0.22	—
1	0.12	1.06	—	—	—	—	—	0.33	—
2	0.37	0.93	—	0.14	0.14	—	—	0.60	—
3	0.63	0.91	—	0.52	0.48	—	—	0.82	—
4	0.78	0.84	—	0.86	0.76	—	—	0.81	—
5	0.80	1.07	—	1.09	0.92	—	—	0.81	0.04
6	0.77	1.00	0.48	1.08	1.00	—	0.05	0.85	0.17
7	0.92	1.00	0.61	1.07	1.00	—	0.11	0.95	0.33
8	0.97	—	0.67	1.05	1.00	0.14	0.21	0.98	0.56
9	1.00	—	0.80	1.03	1.00	0.29	0.36	1.00	0.73
10	—	—	0.89	1.02	—	0.41	0.58	—	0.88
11	—	—	0.95	1.00	—	0.55	0.85	—	0.97
12	—	—	1.08	—	—	0.68	1.14	—	1.00
13	—	—	1.00	—	—	0.90	1.40	—	1.00
14	—	—	—	—	—	0.92	1.56	—	1.00
15	—	—	—	—	—	1.00	1.60	—	1.00
16	—	—	—	—	—	1.00	1.50	—	1.00
17	—	—	—	—	—	1.00	1.28	—	1.00
18	—	—	—	—	—	1.00	1.00	—	—
19	—	—	—	—	—	1.00	1.00	—	—
20	—	—	—	—	—	1.00	1.00	—	—
21	—	—	—	—	—	—	1.00	—	—
22	—	—	—	—	—	—	1.00	—	—
23	—	—	—	—	—	—	1.00	—	—
24	—	—	—	—	—	—	1.00	—	—
25	—	—	—	—	—	—	1.00	—	—
26	—	—	—	—	—	—	1.00	—	—
27	—	—	—	—	—	—	1.00	—	—
28	—	—	—	—	—	—	1.00	—	—
29	—	—	—	—	—	—	1.00	—	—

$$(5)\quad F_{L,t} = F_{L,t-1} + \left(\frac{F_{max} - F_{min}}{T_f}\right) \text{ for } t<T_f,$$

where $F_{L,t}$ is the fishing mortality in year *t* for the oldest age, T_f is the duration (years) of increasing *F* for each scenario, and $F_{L,0} = F_{\min}$ for all scenarios. For the historical scenarios, $F_{\min}$, $F_{\max}$, and T_f were different for each stock (Table 3). For the common *F* scenarios, all stocks had $F_{\min} = 0.1$, $F_{\max} = 1.0$, and T_f = one of the values between 5 and 30 yr, in 5-yr intervals. The purpose of varying T_f in different model runs of the common *F* scenarios was to explore the effects of a wide range of rates of change in fishing intensities (rapid to slow increases in *F*). For each T_f, we calculated the rate of change in *F* per year (i.e. $\Delta F \cdot \text{yr}^{-1} = [(F_{\max} - F_{\min})/T_f]$). In both the common and historical scenarios, we set $F_L = F_{\max}$ after the period of increasing *F* and for the remainder of the simulation (i.e. for all $t \geqslant T_f$).

Estimated Populations

Populations were estimated using VPA in the standard formulation shown below (Gulland 1983, p. 105). First, the number of fish in the oldest (*L*) age class in each year was estimated from

$$(6)\quad N_i = \frac{C_i Z_L^{\text{VPA}}}{F_L\,(1 - e^{-Z_L^{\text{VPA}}})},$$

where $Z_L{}^{\text{VPA}} = F_L + M^{\text{VPA}}$, and F_L for each year was determined from equation (5) above. Thus, we assumed that the true time series of F_L values was known. F_i values for each of the younger ages in each cohort were calculated by finding the solution to

$$(7)\quad \frac{N_{i+1}}{C_i} = \frac{Z_i^{\text{VPA}}\, e^{-Z_i^{\text{VPA}}}}{F_i\,(1 - e^{-Z_i^{\text{VPA}}})},$$

which comes from solving equation (1) for N_i, and substituting the result for the left hand side of equation (6). Equation (7) was solved for F_i using Muller's (1956) method available in the IMSL subroutine ZREAL1 (IMSL 1982). Starting values for the subroutine were estimated from

$$(8)\quad F_i = \log_e(N_i / N_{i+1}) - M^{\text{VPA}},$$

where the N_i was calculated using Pope's (1972) cohort analysis approximation,

$$(9)\quad N_i = N_{i+1} e^{M^{\text{VPA}}} + C_i e^{(M^{\text{VPA}}/2)}.$$

F_i values from solving equation (7) were then used in equation (10) along with M^{VPA} to solve for N_i

$$(10)\quad N_i = N_{i+1} / e^{-Z_i^{\text{VPA}}}.$$

This process was repeated for each cohort, working backwards in time in the usual fashion. We used the iterative method of Rivard (1983) in which new age-specific selection coefficients were calculated from the table of F_i estimates and the VPA was repeated until selection coefficients from successive iterations converged. The age-specific selection coefficients were used in

TABLE 3. For each of the nine stocks studied here, we show from left to right the assumed true instantaneous natural mortality rate, M, and six characteristics of the historical F scenarios: the observed minimum and maximum F ($F_{\min}$ and $F_{\max}$), duration of the period of increasing F, range in cumulative F values between cohorts at the start and end of the period of increasing F, and the rate of change in F per year (unscaled and then scaled relative to M).

Stock	True M	$F_{\min}$	$F_{\max}$	Years of increasing F (T_f)	Range in cumulative F	$\Delta F \cdot \mathrm{yr}^{-1}$	$\Delta F \cdot \mathrm{yr}^{-1} \cdot M^{-1}$
Atlantic mackerel	0.30	0.05	0.85	10	2.074	0.08	0.27
Atlantic menhaden	0.45	0.70	3.00	6	4.359	0.38	0.85
Dover sole	0.20	0.12	0.25	9	0.371	0.01	0.07
Fortune Bay herring	0.20	0.02	1.00	6	1.408	0.16	0.82
Pacific cod	0.65	0.22	0.76	6	0.700	0.09	0.14
Pacific halibut	0.20	0.11	0.30	12	0.591	0.02	0.08
Pacific ocean perch	0.05	0.05	0.35	3	0.022	0.10	2.00
West Scotland herring	0.10	0.20	1.10	8	1.654	0.11	1.12
Yellowfin sole	0.25	0.08	0.29	4	0.040	0.05	0.21

equation (2) to calculate the terminal F values for incomplete cohorts (cohorts that had not yet reached the oldest fished age by the last, or most recent, year of the catch data). F_i values for younger ages of complete cohorts were obtained from equation (7) and remained unchanged after iterations. Thus, the iterative method we used only affected abundance estimates for the incomplete cohorts.

In each case, the time series of F_L's given to the VPA was identical to that used in the simulated population, and the catches were those generated by the simulation. The only error introduced into VPA was through the value of M^{VPA}; we used $M \pm 50\%$. We chose to solve the VPA equations exactly using the IMSL routine because with this range in errors, the incorrect M^{VPA} values for some stocks were outside of the range of acceptable values for either Pope's (1972) or MacCall's (1986) approximations.

Comparison of Estimated and True Abundances

We compared the true abundances with those estimated by VPA using the following index of percent spurious change, P_S,

$$(11) \quad P_S = P_E - P_T,$$

which was the difference between the percentage change in estimated abundances (P_E) over the course of the simulation and the percentage change in true abundances (P_T) over the same period. We used percent change as our index because it was a general measure of the bias that was also proportional to the absolute change per year in recruits or biomass. P_E and P_T were calculated as

$$(12) \quad P_E = \frac{NE_t - NE_1}{NE_1} \cdot 100$$

and

$$(13) \quad P_T = \frac{NT_t - NT_1}{NT_1} \cdot 100,$$

where NT_1 and NE_1 were the true and estimated initial abundances at time $t=1$, respectively, and NT_t and NE_t were the stabilized abundances following the period of increasing F. The latter abundances occurred at time $t=T_f$ for recruitment, and $t=T_f+k-1$ for biomass, where k is the number of ages fished for each stock. We generated catch data for T_f+2k-2 yr to ensure that stabilized abundances were estimated from cohorts that had reached the oldest fished age. Thus, we only used the abundance estimates obtained from "complete" cohorts in our calculations.

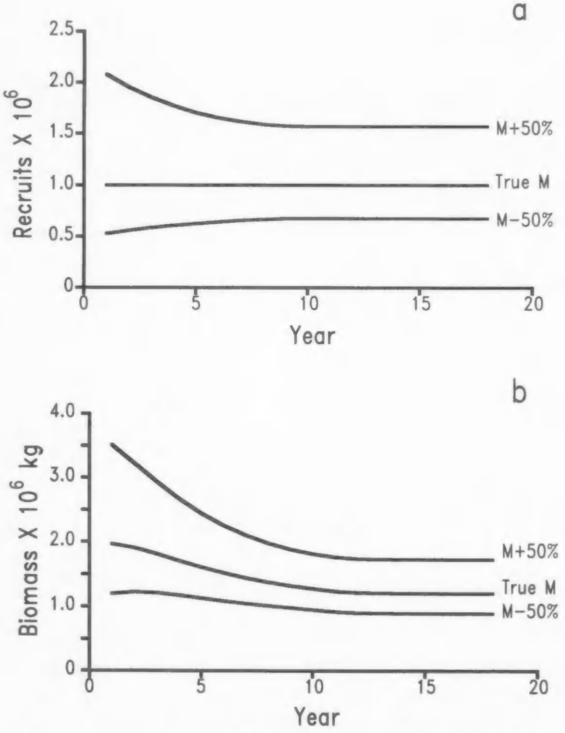

FIG. 1. Time series of Atlantic mackerel recruits and total stock biomass using the historical F trend for that stock. Part (a) shows true recruits (middle line) and recruits estimated by VPA when the natural mortality rate used in VPA (M^{VPA}) was 50% above the true value, M, (top line) or 50% below M (bottom line). Part (b) shows true stock biomass (middle line) and biomass estimated when M^{VPA} was 50% above M (top line) or 50% below M (bottom line). VPA estimation proceeded from the right side of the figure to the left.

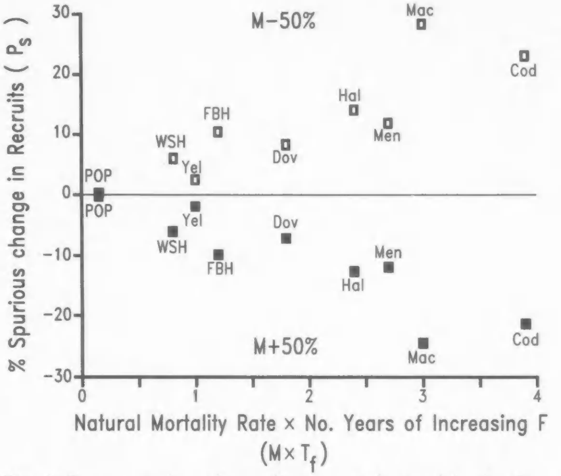

FIG. 2. Percent spurious change (increase or decrease) in abundance of recruits estimated by VPA when M^{VPA} was 50% below M (top half; $r^2=0.78$, $P<0.002$) or 50% above M (bottom half; $r^2=0.81$, $P<0.002$). Stock abbreviations are defined in Table 1. For each stock, the true M value, duration of the period increasing F (T_f), F_{min} and F_{max} are for the historical scenarios (Table 3).

We refer to P_T and P_E as the true and estimated change in abundance and P_S as the spurious change or spurious trend. We calculated each of these three quantities for both recruits and stock biomass. In the case of recruitment, $P_S=P_E$ because P_T, the true trend, was 0 (i.e. we assumed constant recruitment in our simulations). Thus, in our results below, $P_S>0$ for recruitment meant that VPA estimated a false increasing time trend in recruits, whereas $P_S<0$ would come from a spurious decreasing trend. However, for stock biomass, increases in F cause a decline in both the simulated (i.e. true) and estimated biomass as older age classes are depleted from the initial stock. Thus, $P_S>0$ for stock biomass meant that VPA underestimated the true percentage decline in biomass. For example, $P_E=-20\%$ for biomass and $P_T=-30\%$ gives $P_S=+10\%$ from equation (11). In contrast, $P_S<0$ indicated an overestimate of the decline in biomass.

Results

Historical F Scenarios

Figure 1 shows results for Atlantic mackerel (*Scomber scombrus*) at various M^{VPA} values when the historically observed F scenario was applied. When M^{VPA} equalled the true M, VPA correctly estimated the true recruitment and true stock biomass (middle lines in Figs. 1a and 1b). Therefore, trends in F do not cause VPA to estimate abundance incorrectly when M is known exactly and there are no other errors in VPA inputs. However, when M^{VPA} was incorrect, estimates of recruits and stock biomass diverged from the true values as VPA proceeded back in time through the cohorts (from right to left in Fig. 1). When M^{VPA} was less than M, recruitment and biomass were both underestimated, VPA generated a false increasing time trend in recruits, and underestimated the decreasing trend in biomass (Fig. 1). When M^{VPA} was greater than M, VPA generated spurious decreasing time trends in recruitment, overestimated the percent decline in biomass, and overestimated recruitment and stock biomass (Fig. 1). We found similar results for other stocks; for a given sign of error in M^{VPA}, the spurious change in recruits and biomass for the historical scenarios increased with the product of M and the number of years of increasing F, T_f (Figs. 2 and 3). When M^{VPA} was incorrect, the percent spurious change was greater for recruits than for total stock biomass for a given stock (Figs. 2 and 3).

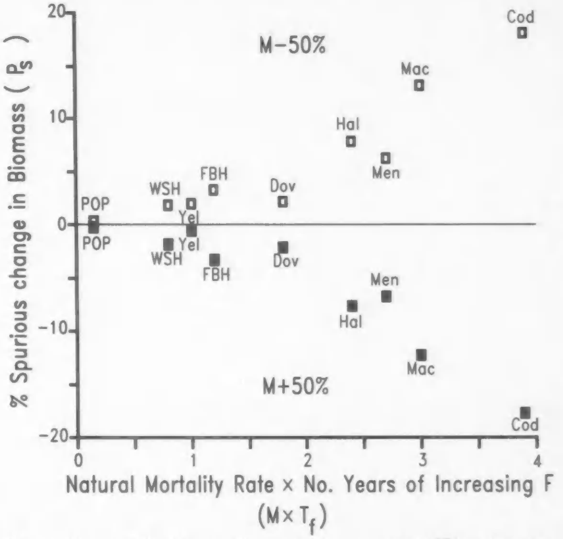

FIG. 3. Same as Fig. 2 except for stock biomass. For $M^{VPA}=M-50\%$, $r^2=0.86$, $P<0.001$ and for $M^{VPA}=M+50\%$, $r^2=0.88$, $P<0.001$.

Explanation of Historical F Scenarios

The underlying cause of the time-trend bias is a simple extension of the well-known effects of errors in VPA parameters (Pope 1972; Agger et al. 1973; Ulltang 1977; Sims 1984; Bradford and Peterman 1989; Sampson 1988; Hilden 1988). Pope (1972) showed that for a given M^{VPA}, errors in estimated abundance at a given age caused by an incorrect choice of $F_L{}^{VPA}$ decreased as the cumulative fishing mortality on the cohort increased. Pope's analysis was extended to include errors in M by Sims (1984), who gave an approximate formula that related the relative error in abundance estimates to the magnitude of error in M^{VPA} and cumulative fishing mortality (see Sims' equation 8). These results imply that identical successive annual cohorts that experience different cumulative fishing mortality rates will have different amounts of bias in recruitment estimates for each successive cohort when these estimates are derived from VPA with an incorrect $F_L{}^{VPA}$ or M^{VPA}.

Indeed, this is the underlying cause of the spurious time trends in abundance estimates shown in this paper; differences in cumulative fishing mortality rate exist among successive cohorts because of changes in F over time and these are coupled with an incorrect input parameter to VPA, M^{VPA}. This mechanism is detailed below.

A full explanation of the time-trend bias in Fig. 1 requires several steps. (1) For the case of $M^{VPA}=M+50\%$ in Fig. 1a, recruitment estimates in years 10 to 18 were constant but biased above true recruitment; this overestimate was due solely to the error in M^{VPA} because there was no change in F during this period. (2) However, the sequence of fishing mortality rates

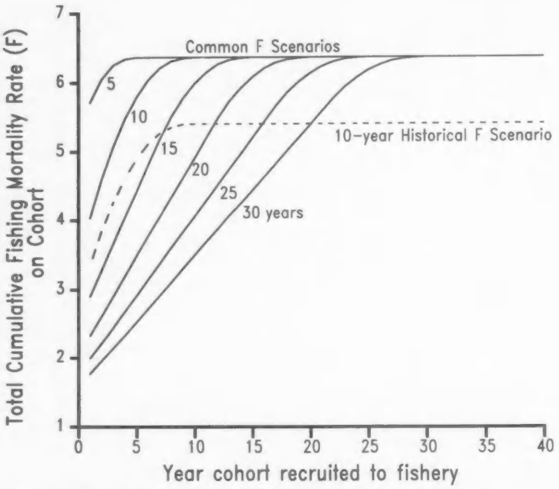

FIG. 4. Total fishing mortality rate (ΣF_i) experienced by each cohort of Atlantic mackerel in simulations using true M and F_L values. Lines are shown for each of the simulated common F scenarios (solid lines) ranging in duration from 5 to 30 yr, and for the 10-yr historical F scenario (dashed line). VPA estimation proceeded from the right side of the figure to the left.

during the period of increasing F caused cohorts that recruited to the fishery in years 1–9 to experience lower cumulative fishing mortality rates (ΣF_i for all fished ages) than cohorts that recruited later (dashed line in Fig. 4). As Sims (1984) noted, the amount of error in an abundance estimate resulting from an error in M^{VPA} is larger when the cumulative fishing mortality experienced by a cohort is small. Thus, the lower cumulative fishing mortality rates on cohorts that recruited in years 1–9 led to more biased abundance estimates in those years. (3) Finally, recruits from each successive cohort that recruited in years 1–9 were estimated with decreasing bias, because as F increased, cumulative fishing mortality rates on each successive cohort increased (Fig. 4 dashed line). In other words, the error in abundance estimates already present due to an incorrect M^{VPA} was exaggerated most in early years when cumulative F was low and least when cumulative F was high, at the end of the period of increasing F. Hence, the result was a time trend in estimated recruitment (Fig. 1a), despite a constant true recruitment and the use of the true time series of F_L by VPA. For brevity, we refer below to this mechanism that explains time trends as "differences in cumulative fishing mortality rate among successive cohorts." In the case of $M^{VPA} = M - 50\%$, the explanations are similar but errors appear as underestimates of true abundance rather than overestimates (bottom of Fig. 1a).

Time trends occur in stock biomass estimates (Fig. 1b) through the same mechanisms as in recruits. However, spurious time trends were generally greater for recruits than for stock biomass because estimates of abundance for younger ages will be more biased in percentage terms than estimates for older ages within the same cohort; as VPA works back through a cohort, these estimates of young ages result from more applications of the incorrect Z^{VPA} in equation (10). Total stock biomass estimates are less biased than recruits because biomasses are the weighted sum of abundances over ages, and the less biased estimates for older ages dilute the effect of the more biased abundance estimates of younger ages.

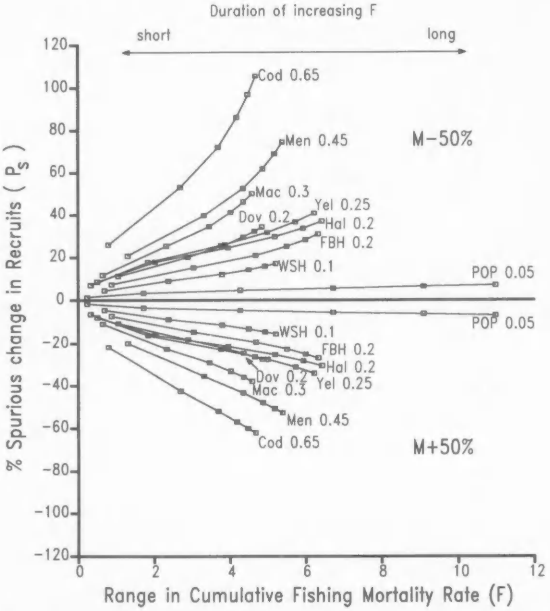

FIG. 5. Percent spurious change (increase or decrease) in abundance of recruits estimated by VPA when M^{VPA} was 50% below M (top half) or 50% above M (bottom half). Curves are for the stocks abbreviated on the right (defined in Table 1) and true M values are beside stock abbreviations. For each stock, the duration of the common F scenarios ranged from 5 yr (left point) to 30 yr (right point) in 5-yr intervals. "Range in cumulative fishing mortality rate" is the maximum cumulative F on cohorts minus the lowest cumulative F encountered in that F scenario (see text).

Spurious change in recruits and biomass increased with the product of M and T_f (Figs. 2 and 3) because for any given stock, longer periods of increasing F result in greater differences in cumulative fishing mortality among successive cohorts (Fig. 4), and because for any given cumulative F, larger M^{VPA} results in more bias (see section "Explanation of comparisons among stocks" below). Thus, $M \cdot T_f$ is an approximate index of the time-trend bias caused by an incorrect M^{VPA} for these historical situations. The index is not perfect because of differences in historical ranges in F and partial recruitment vectors among stocks (Tables 2 and 3).

Common *F* Scenarios

Comparisons among stocks

One purpose of the common F scenarios was to compare spurious time trends among stocks with different life history characteristics. This was not possible in the previous scenarios because of historical differences in F trends among stocks; in this section, we applied the same sequence of increasing F to all stocks. In these common F scenarios, F_{min} and F_{max} were the same for all durations of increasing F, ranging from 5 to 30 yr, as described above. Because time trends in abundance estimates caused by increasing trends in F in conjunction with an incorrect M^{VPA} result from differences in cumulative fishing mortality among successive cohorts, we calculated a single index of these differences, which was the cumulative fishing mortality rate on cohorts that recruited to the fishery in the year

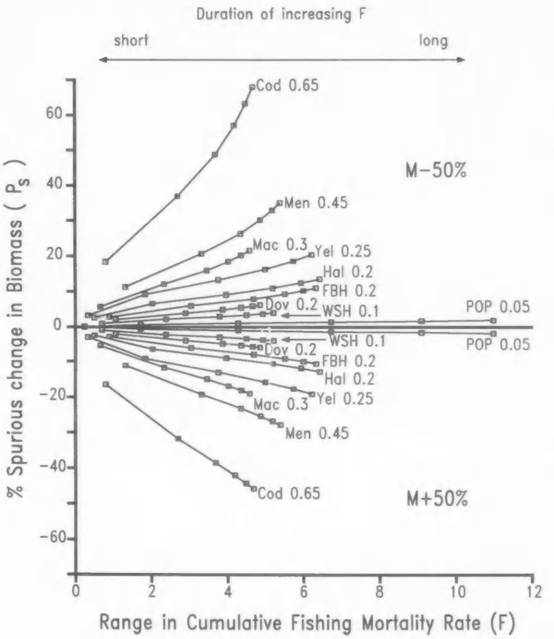

FIG. 6. Same as Fig. 5 except for stock biomass.

T_f (when $F_L = F_{max}$) minus cumulative F on cohorts that recruited in year 1 (top minus bottom values on a given curve in Fig. 4). This index was specific to the stock and the number of years of increasing F because stocks had varying numbers of exploited age classes and different age-specific selection coefficients and because for any given stock the cumulative fishing mortality on cohorts varies with the numbers of years of increasing F (Fig. 4). We henceforth call this index ''the range in cumulative F.''

We used the range-in-cumulative-F index to compare the percent spurious change in recruits (Fig. 5) and stock biomass (Fig. 6) across stocks. For each stock, six square points are shown. Each point on a given curve is for a given duration of increasing F, ranging from 5 yr (left-most point on each curve) to 30 yr (right-most point). Long periods of increasing F resulted in the largest absolute values of percent spurious change in abundance estimates within a given stock. Stocks with larger M (e.g. Pacific cod (*Gadus macrocephalus*), menhaden (*Brevoortia tyrannus*)) had greater spurious trends for a given length of increasing-F period than stocks with low M. Trends for high M stocks were also more sensitive to changes in the length of the increasing-F period; high M stocks had a much larger increase in the absolute value of percent spurious change than low M stocks when the rate of change in F was varied from rapid (left ends of curves) to slow (right ends). Three stocks (i.e. Pacific halibut (*Hippoglossus stenolepis*), Dover sole (*Microstomus pacificus*) and yellowfin sole (*Limanda aspera*)) with similar M values had overlapping curves of percent spurious change in recruits. As found for the historical scenarios, recruits had larger absolute values of percent spurious change than biomass for a given situation.

Explanation of comparisons among stocks

First, within each stock, longer periods of increasing F caused larger absolute values of the percent spurious change in abundance estimates than short periods, giving slopes to the curves in Figs. 5 and 6. This was because we assumed F_{min} and F_{max} were constant; the longest durations thus create the lowest cumulative F on cohorts present during the increasing-F phase (e.g. Fig. 4 for mackerel) and as noted above, low cumulative F's give the largest errors in estimated abundance. In the Sensitivity Analysis section below, we remove the assumption of a constant F_{max} and results are similar. Stocks with high M have greater spurious time trends ($|P_S|$) than low M stocks for a given range in cumulative F because errors in abundance estimates for these stocks are inflated more by the larger Z^{VPA} (equation (10)) than estimates for low M stocks.

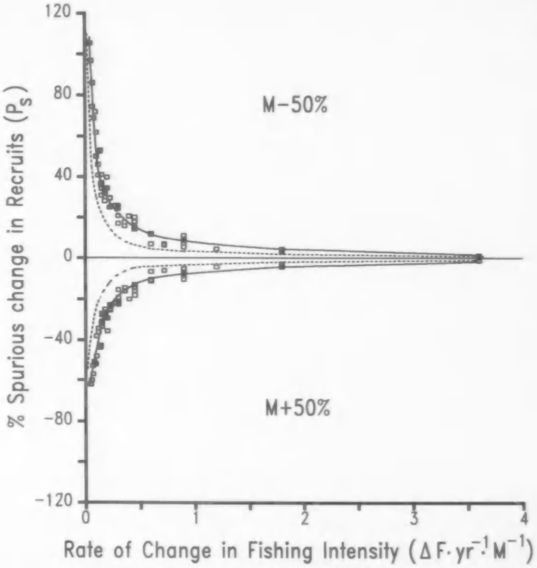

FIG. 7. Percent spurious change in abundance of recruits estimated by VPA in relation to the relative rate of change in fishing intensity (equal to the rate change in F_L per year divided by the true M value for each stock). Top half is for M^{VPA} 50% below M and bottom half is for M^{VPA} 50% above M. Each point in each half represents one stock and one duration of increasing F, with the shortest durations on the right side. Points and outer curves are for F_L ranging from 0.1 to 1.0. Inner dashed curves without points are for F_L ranging from 0.05 to 0.5.

Similarly, stocks with large M have steeper slopes in Figs. 5 and 6 than stocks with low M (i.e. the former are more sensitive to changes in the duration of the increasing-F phase) because in successive applications of equation (10) in the backwards recursion, the change in errors in N_i resulting from the change in range of cumulative fishing mortality is exaggerated more by the larger Z^{VPA} of large M stocks.

Generalization across stocks

The second purpose of the common F scenarios was to permit generalization of results across stocks. To generalize our findings, we used the variable defined above, $\Delta F \cdot yr^{-1} \cdot M^{-1}$, which is a measure of the rate of change in intensity of F, relative to M. When the results from the common F scenarios for all stocks were combined, we found an inverse relationship between percent spurious change in recruits and this relative rate of change in intensity of exploitation (outer curves and symbols in Fig. 7). However, the same plot for percent spurious change in biomass (Fig. 8) gave a family of curves; stocks with the lowest M values tended to have smaller percent spurious changes. In

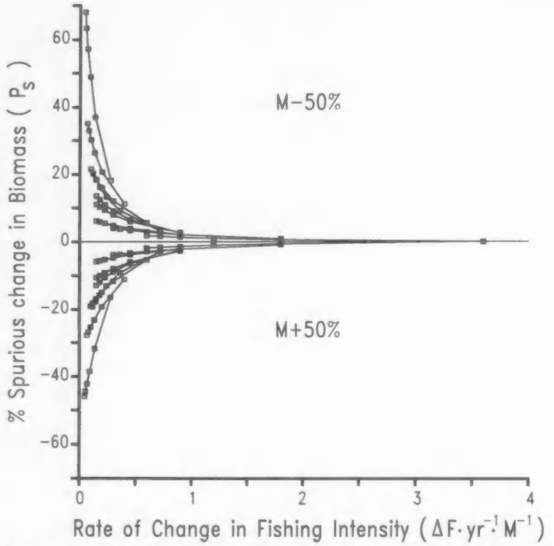

FIG. 8. Same as Fig. 7 except for stock biomass and larger F_L range only. Points of different durations of increasing F are connected for a given stock. Curves for high M stocks have steepest slopes; low M stocks tend to have shallow sloped curves.

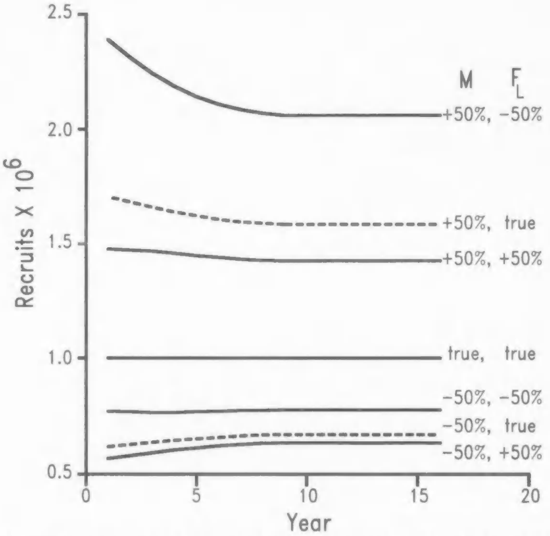

FIG. 9. True recruits (horizontal line) and recruits estimated by VPA under various combinations of errors in M^{VPA} and $F_L{}^{VPA}$, for the historical F scenario applied to Dover sole. Dashed curves are for no error in $F_L{}^{VPA}$ in conjunction with M^{VPA} overestimated (top) or underestimated (bottom) by 50%. Curves for errors in M^{VPA} in conjunction with errors in $F_L{}^{VPA}$ of ±50% of the true F_L bracket each related dashed curve.

both Figs. 7 and 8, the smallest spurious trends came from cases with rapid increases in F.

Explanation of generalization across stocks

The general patterns in Figs. 7 and 8 result from a combination of processes described in previous sections. We noted above why longer periods of increasing F caused larger absolute values of percent spurious change in abundance estimates. Because long periods of increasing F lead to a slow rate of change in F ($\Delta F \cdot yr^{-1}$) in our common F scenarios, the spurious trends in recruits resulting from an incorrect M^{VPA} are largest at the slowest relative rate of change in fishing intensity (Fig. 7).

Biomass trends for the nine stocks showed a similar qualitative pattern (Fig. 8). Again recall that periods of increasing F cause decreasing trends in true biomass as the older age classes are depleted, and therefore the percent spurious change in biomass is the difference between the true and estimated trends (equation (11)). True percentage decreases in biomass, (P_T), were larger for stocks with low M because, (1) all stocks shared the same F scenarios, (2) F constituted a larger portion of Z for these low M cases than for high M stocks, and (3) stocks with low M therefore experienced relatively greater percentage changes in Z as F increased compared with stocks with high M. However, these large true trends were exaggerated less by VPA in stocks with low M than in stocks with high M because low M stocks have a small Z^{VPA} in equation (10). The net result is that the absolute value of the percent spurious change ($|P_S| = |P_E - P_T|$) is smaller for stocks with lower M. While this is the same qualitative pattern as found for trends in recruitment, biomass trends are also affected by other factors, such as differences in the number of fished ages and the weight-at-age vector, which are not captured by our simple measure of relative rate of change in fishing intensity. This is particularly true at low rates of change in F and it leads to the family of curves in Fig. 8.

Sensitivity Analyses

Changes in assumed F scenarios

We explored the robustness of our results to changes in some of our assumptions. First, we examined how the absolute range in F_L during the increasing F phase influenced our common F scenario results. Previously, we set $F_{min} = 0.1$ and $F_{max} = 1.0$; here we halved that range (0.05 and 0.5) to generate less intensive exploitation. For a given stock, percent spurious changes in recruits were generally smaller for the lower F_L range, and generalized results across stocks and across durations of increasing F were similar to those for the larger F_L range (inner dashed curves in Fig. 7). The smaller range in F_L created smaller differences in cumulative F among successive cohorts, creating smaller spurious time trends than with the large range. These results are distinct from the effect of F_L values on absolute N_i estimates, which become less biased with large cumulative F (Pope 1972).

Second, in the common F scenarios above we assumed a fixed F_{min} and F_{max} and varied the rate of increase in F by changing the number of years of increasing F. In a sensitivity analysis, we simulated an alternative form of F trend where F_{min} was fixed and F_L increased by a fixed amount each year, resulting in a different F_{max} for each duration of the increasing-F period. The results from these runs had the same qualitative pattern as in Figs. 5 and 6; a longer period of increasing F resulted in greater percent spurious change for the same reason as it did in the main results: it generated a wider range in cumulative F values among cohorts than shorter durations.

Changes in assumed errors

In our simplified analyses in previous sections, we assumed that the time series of F_L was known for doing VPA, but if errors were made in that input, the resulting spurious time trends would be different from those we have shown. We therefore added errors in ${F_L}^{\mathrm{VPA}}$ of $\pm 50\%$ of the true F_L. Simultaneous errors in M^{VPA} and ${F_L}^{\mathrm{VPA}}$ either tended to reduce or exaggerate the effects of the error in M^{VPA} alone, depending on the signs of the two errors (e.g. Dover sole in Fig. 9). This was true of both the absolute error in N_i estimates and the percent spurious change for recruits and stock biomass. Trends due to errors in ${F_L}^{\mathrm{VPA}}$ arise through the same mechanisms (differences in cumulative mortality rate among cohorts) noted previously for errors in M^{VPA}. Because errors in M^{VPA} and ${F_L}^{\mathrm{VPA}}$ are unlikely to cancel one another exactly, some spurious time trend will usually result in practice; its magnitude will depend on the relative magnitudes and signs of errors in M^{VPA} and ${F_L}^{\mathrm{VPA}}$.

We also considered a fixed absolute error in M^{VPA} (e.g. $M \pm 0.1$) rather than a fixed percentage error and again found the same qualitative pattern as in Figs. 5 and 6 of increasing magnitude of spurious change with longer duration of the period of increasing F.

Decreasing recruitments

To simplify our analysis and explanations, we assumed previously that the decreases in stock biomass that occurred as a result of increasing F did not affect recruitment. This was consistent with the marine fish literature, much of which shows that it is very difficult to detect any response of recruitment to changes in spawning stock biomass over the ranges of observed biomasses (reviewed by Rothschild 1986). For our sensitivity analysis, we examined common F scenario situations in which recruitment decreased each year by a given percentage, paralleling the decrease in stock biomass. When compared with the constant recruitment cases presented above, percent spurious time trends that occurred with decreasing recruitment were smaller by at most one-third (for both recruits and biomass) because decreasing trends occurred in both true and estimated abundances. These results were independent of the direction of error in M^{VPA}.

These sensitivity runs clearly show that spurious time trends in abundances reconstructed by VPA are likely to occur under a wide range of conditions when increasing trends in F are present along with an error in one of the parameters input to VPA.

Discussion

Our results show that in the presence of an error in M^{VPA} and an increasing trend in F_L, VPA generates a spurious time trend in both estimated recruits and stock biomass, even if the actual trend in F_L is known. The magnitude of these false trends is greatest when, for a given M and range in F_L, F increases over a long period, that is, when the percentage change in F_L per year is small (Figs. 7 and 8). This counterintuitive result arises because a slow rate of change in F causes relatively low cumulative F on early cohorts and a wider range in cumulative fishing mortality rates than do rapid F increases, and low cumulative F in turn results in more biased abundance estimates for those early cohorts.

We performed our simulations with a time series of F_L values that approximated the trends in F_L observed historically. However, these observed trends in F are affected by the very bias that we document in this paper; most published tables of F estimates for our stocks came from VPA. Thus, the true rate and magnitude of increasing F trends in our historical scenarios may have been over- or underestimated, and the magnitude of the percent spurious changes may be incorrect for those specific scenarios. Nevertheless, the remainder of our conclusions from using the "common F scenarios" are valid; with any time trend in F_L and an error in M^{VPA}, spurious time trends will be embedded in the resulting abundance estimates.

In our simulations, we assumed that increasing trends in F started at the beginning of the time series of the catch data. If we had generated catch data for years prior to the start of an increase in F, and assumed that F was constant at a minimum level during those years, VPA estimates of recruits and biomass would have continued to diverge from the true values (e.g. Fig. 1) as VPA proceeded back through the cohorts until the cumulative fishing mortalities of successive cohorts were identical (i.e. until the cumulative F on recruits reached a constant minimum level off to the left side of Fig. 4.). The estimated abundance for those early years would therefore be constant. Thus, under such conditions, the spurious trends caused by increasing F would be larger than we have reported here. However, for different durations of the increasing-F period, the estimated abundance in the early years would approach the same plateau of constant bias and therefore the same percent spurious change would result. Such a situation could arise in practice if reliable age-specific catch data were available prior to the start of a time trend in F. However, this may be unlikely because the stimulus for starting to gather data often occurs well into the fishing-up phase in development of a fishery. Even if such data were available, spurious time trends would still exist and the effect of M would be as shown previously. The only change from our results above would occur when comparing two identical stocks that had different durations of increasing F, and where data were available for the period prior to the start of the time trend in F. However, our conclusions concerning the effect of different durations of increasing F will hold whenever longer periods of increasing F result in a greater range in cumulative F (e.g. Figs. 5 and 6).

The spurious time trends that we have documented occur in a wider variety of situations than has been shown previously. First, Hilden (1988) documented false time trends in recruitment in the presence of increasing F trends and an error in M, but he made the additional assumption of an underlying stock–recruitment relationship. In our main analysis, we assumed that no change in recruitment occurred over the range of stock biomasses observed during the simulated periods of increasing F, yet we also found spurious trends due to differences in cumulative F among successive cohorts.

Second, the spurious time trends documented by Bradford and Peterman (1989) arose from using catch data in recent years that included incomplete cohorts (year-classes that had not reached the terminal age by the last year of the catch data), and from using "noniterative" VPA, that is, when the partial recruitment vector of age-specific selectivity coefficients was estimated empirically and the VPA was run only once. Analysts use VPA in this way when major changes in catchability have occurred during the period (e.g. Winters et al. 1985). However, we did not include incomplete cohorts in the simulated catch data here and we used the more common "iterative" VPA (Rivard 1983) instead of noniterative VPA. Our spurious trends resulted from a different and more common mechanism (iterative VPA with increasing F trends and an incorrect M^{VPA}) than the trends in Bradford and Peterman (1989) (noniterative VPA with incomplete cohorts and an incorrect M^{VPA} or ${F_L}^{\mathrm{VPA}}$). Our results are therefore more general than those of Bradford and Peterman (1989) and Hilden (1988) because false trends will occur even if all incomplete cohorts are removed from the input catch data, even if iterative VPA is used, and without any assumed effect of stock biomass on recruitment.

If noniterative VPA is used with catch data sets that include both incomplete cohorts and periods of increasing F, noniterative VPA will either tend to exaggerate the spurious time trend already documented above due to increasing F trends alone, or will result in spurious trends in the opposite direction, again depending on the signs of errors in M^{VPA} and $F_L{}^{\text{VPA}}$ (Bradford and Peterman 1989). Thus, the potential exists for the introduction of complex, nonmonotonic spurious time trends into reconstructed abundances.

We have only addressed increasing F trends but our qualitative results apply more generally to any case in which F tends to increase or decrease (with some variability) over a given period. In the case of a decrease in F, estimates of abundance will converge toward the true value as VPA proceeds back in time through the cohorts, and spurious trends will be opposite in sign from those reported here.

General Implications

These results have considerable relevance for two reasons. First, in practice, M^{VPA} is unlikely to equal the true M. Methods for estimating M for fish populations usually report large confidence intervals (Alverson and Carney 1975; Pauly 1980; Roff 1984; Gunderson and Dygert 1988) or large ranges in different estimates of M for the same species (Vetter 1988), partly because of confounding with other parameters such as the catchability coefficient (Paloheimo 1980). Second, VPA has commonly been used on catch data sets that include periods of increasing F (e.g. references in Table 1 except Fournier (1983) and Archibald et al. (1983)). Thus, the presence of periods of increasing or decreasing trends in fishing mortality rate in past data sets used in VPA may have introduced biases that influenced interpretations of the success or failure of past management actions. As well, studies of mechanisms of variability in recruitment estimated by VPA (e.g. Shepherd et al. 1984; Koslow et al. 1987; Hollowed et al. 1987) and stock–recruitment relations may also have been biased, possibly to the point of masking relationships that were present in nature (Hilden 1988) or creating spurious correlations. Analysts should therefore generate different time series of recruits and stock biomass for each set of parameter estimates used in a VPA. A separate correlation should be performed between the independent variate (such as an environmental factor) and each set of abundance data. Given the natural variability in recruitment and M in nature, and the possibility of time trends in M, it is unlikely that such exercises will identify the true underlying correlations; however, comparisons of results of these different reconstructed time series will demonstrate the sensitivity of such correlations to assumed VPA parameter estimates.

The spurious time trends in abundance estimates caused by increasing F trends may cause problems for some VPA tuning methods, particularly those which use regressions of survey CPUE (catch per unit effort) on VPA estimates (e.g. Rivard 1983; rho method of Pope and Shepherd 1985). Depending on the extent of the F trend bias, the slope from such regressions (i.e. the catchability coefficient) will be biased due to spurious time trends in the estimates from VPA. Therefore, we concur with Mohn (1983) and Pope and Shepherd (1985), who suggest that tuning methods that use effort data are preferable to those that use CPUE data.

Similarly, if spurious time trends in recruitment estimates are present, total allowable catch (TAC) projections will be biased if they are based partly on, (1) projected recruitment from regressions of recruit survey CPUE on VPA recruit estimates (Brander 1987), (2) regressions of VPA recruitment estimates on time (Rivard 1983), or (3) long-term average recruitment (Rivard and Foy 1987). For $M^{\text{VPA}}<M$, projected recruitment and biomass will generally be underestimated, there will be a spurious increasing trend in recruitment, and the estimated decrease in biomass will be less than the true decrease. In contrast, for $M^{\text{VPA}}>M$, projected recruitment and biomass will be overestimated, recruitment will have a spurious decreasing trend, and the estimated decrease in stock biomass will be greater than the true decrease.

The well known problems with VPA have stimulated the development of nonsequential, statistical fitting methods of estimating abundance, which are now gaining wider acceptance as plausible alternatives to VPA (e.g. Doubleday 1976; Fournier and Archibald 1982; Collie and Sissenwine 1983; Deriso et al. 1985; Gudmundsson 1986; Methot 1989). These methods may be more robust to errors in input parameters, particularly F_L, because they commonly use auxilliary data to help determine estimates. However, most fitting methods require as input an assumed and constant M value and for those which permit estimation of M, M estimates are poorly determined (e.g. Fournier 1983) and very sensitive to errors in other inputs (e.g. aging errors; Fournier and Archibald 1982). Thus, while it is clear that nonsequential techniques will produce biased abundance estimates when M is incorrect, it is not clear whether errors in input parameters will lead to spurious time trends in estimates obtained from such techniques. Therefore, analysts must determine how sensitive abundances estimated by the new fitting methods are to errors in input information. The history of VPA work clearly demonstrates the necessity of such sensitivity analyses; in recent years, analytical and numerical analysis techniques have shown that under certain commonly found circumstances, VPA outputs are quite sensitive to errors in inputs and assumptions (e.g. Ulltang 1977; Sims 1984; Rivard and Foy 1987; Sampson 1987; 1988; Kimura 1989; Bradford and Peterman 1989). These same techniques should be used in rigorous evaluations of the new statistical fitting methods that some researchers hope will replace VPA. Some evaluations have been done (e.g. Deriso et al. 1989) but they should also include cases in which trends in F occur.

Regardless of which stock reconstruction method analysts use, our results and others noted above emphasize the importance of presenting to fishery managers the results of sensitivity analyses when parameters are uncertain. Such information is essential to enable managers to make the best possible decisions, given the large uncertainty in our understanding of these systems. As well, it is essential that more effort be directed at obtaining more precise and accurate estimates of data used in analyses, especially key parameters such as M, because no stock reconstruction method can completely avoid bias if parameter estimates are poor.

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References

AGGER, P., I. BOETIUS, AND M. LASSEN. 1973. Error in the virtual population analysis: the effect of uncertainties in the natural mortality coefficient. J. Cons. Int. Explor. Mer 35: 93.

ALVERSON, D. L. AND M. J. CARNEY. 1975. A graphical review of the growth and decay of population cohorts. J. Cons. Int. Explor. Mer 36: 133–143.

ANDERSON, E. D., AND A. J. PACIORKOWSKI. 1980. A review of the northwest Atlantic mackerel fishery. Rapp. P.-V. Reun. Int. Explor. Mer 177: 175–211.

ARCHIBALD, C. P., D. FOURNIER, AND B. M. LEAMAN. 1983. Reconstruction of stock history and development of rehabilitation strategies for Pacific ocean perch in Queen Charlotte Sound, Canada. N. Am. J. Fish. Manage. 3: 283–294.

ARENHOLZ, D. W., W. R. NELSON, AND S. P. EPPERLY. 1987. Population and fishery characteristics of Atlantic menhaden, (*Brevoortia tyrannus*). Fish. Bull. 85: 569–600.

BRADFORD, M. J., AND R. M. PETERMAN. 1989. Incorrect parameter values used in virtual population analysis (VPA) generate spurious time trends in reconstructed abundances. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and on evaluation of parameters used in stock assessment. Can. Spec. Publ. Fish. Aquat. Sci. 108 (In press)

BRANDER, K. 1987. How well do working groups predict catches? J. Cons. Int. Explor. Mer 43: 245–252.

COLLIE, J. S., AND M. P. SISSENWINE. 1983. Estimating population size from relative abundance data measured with error. Can. J. Fish. Aquat. Sci. 40: 1871–1879.

DERISO, R. B., P. R. NEAL, AND T. J. QUINN II. 1989. Further aspects of catch-age analysis with auxiliary information. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and on evaluation of parameters used in stock assessment. Can. Spec. Publ. Fish. Aquat. Sci. 108 (In press)

DERISO, R. B., T. J. QUINN II, AND P. R. NEAL. 1985. Catch-age analysis with auxiliary information. Can. J. Fish. Aquat. Sci. 42: 815–824.

DOUBLEDAY, W. G. 1976. A least squares approach to analyzing catch at age data. Int. Comm. Northw. Atlant. Fish. Res. Bull. 12: 69–81.

ESPINO, M. A., AND C. WOSNITZA-MENDO. 1986. Peruvian hake fisheries from 1971 to 1982. Calif. Coop. Oceanic Fish. Invest. Rep. 27: 113–120.

FOURNIER, D. A. 1983. An analysis of the Hecate Strait Pacific cod fishery using an age-structured model incorporating density-dependent effects. Can. J. Fish. Aquat. Sci. 40: 1233–1243.

FOURNIER, D. A., AND C. P. ARCHIBALD. 1982. A general theory for analyzing catch at age data. Can. J. Fish. Aquat. Sci. 39: 1195–1207.

FRANCIS, R. 1986. Two fisheries biology problems in west coast groundfish management. N. Am. J. Fish. Manage. 6: 453–462.

GULLAND, J. A. 1983. Fish stock assessment. Wiley-Interscience. Chichester, U.K. 223 p.

GUNDERSON, D. R., AND P. H. DYGERT. 1988. Reproductive effort as a predictor of natural mortality rate. J. Cons. Int. Explor. Mer 44: 200–209.

GUNDMUNDSSON, G. 1986. Statistical considerations in the analysis of catch-at-age observations. J. Cons. Int. Explor. Mer 43: 83–90.

HAYMAN, R. A., A. V. TYLER, AND R. L. DEMORY. 1980. A comparison of cohort analysis and catch per unit effort for Dover sole and English sole. Trans. Am. Fish. Soc. 109: 35–53.

HIGHTOWER, J. E., AND G. D. GROSSMAN. 1985. Comparison of constant effort harvest policies for fish stocks with variable recruitment. Can. J. Fish. Aquat. Sci. 42: 982–988.

HILDEN, M. 1988. Errors of perception in stock and recruitment studies due to wrong choices of natural mortality rate in virtual population analysis. J. Cons. Int. Explor. Mer 44: 123–134.

HOAG, S. H., AND R. S. MCNAUGHTON. 1978. Abundance and fishing mortality of Pacific halibut, cohort analysis, 1935–1976. Int. Pac. Halibut Comm. Sci. Rep. 65, 45 p.

HOLLOWED, A. B., K. M. BAILEY, W. S. WOOSTER. 1987. Patterns in recruitment of marine fishes in the northeast Pacific Ocean. Biol. Oceanogr. 5: 99–131.

IMSL. (INTERNATIONAL MATHEMATICAL AND STATISTICAL LIBRARY). 1982. International mathematical and statistical library reference manual. IMSL, Houston, TX.

KIMURA, D. K. 1989. Variability in estimating catch-in-numbers-at-age and its impact on cohort analysis. *In* R. J. Beamish and G. A. McFarlane [ed.] Effects of ocean variability on recruitment and on evaluation of parameters used in stock assessment. Can. Spec. Publ. Fish. Aquat. Sci. 108 (In press)

KOSLOW, J. A., K. R. THOMPSON, AND W. SILVERT. 1987. Recruitment to northwest Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks: influence of stock size and climate. Calif. Coop. Oceanic Fish. Invest. Rep. 18: 136–148.

MACCALL, A. D. 1976. Density dependence of catchability coefficient in the California Pacific sardine, *Sardinops sagax caerula*, purse seine fishery. Calif. Coop. Oceanic Fish. Invest. Rep. 18: 136–148.

1986. Virtual population analysis (VPA) equations for nonhomogeneous populations, and a family of approximations including improvements on Pope's cohort analysis. Can. J. Fish. Aquat. Sci. 43: 2406–2409.

METHOT, R. D. 1989. Synthetic estimates of historical abundance and mortality for northern anchovy, *Engraulis mordax*. *In* E. F. Edwards and B. A. Megrey [ed.] Mathematical analysis of fish stock dynamics: reviews, evaluations and current applications. Am. Fish. Soc. Symp. Ser. No. 6. American Fisheries Society, Bethesda, MD. (In press)

MOHN, R. K. 1983. Effects of error in catch and effort data on tuning cohort analysis, with a postscript on logistic production models, p. 141–150. *In* W. G. Doubleday and D. Rivard [ed.] Sampling commercial catches of marine fish and invertebrates. Can. Spec. Publ. Fish. Aquat. Sci. 66.

MULLER, D. E. 1956. A method for solving algebraic equations using an automatic computer. Mathematical Tables and Other Aids to Computation 10: 208–215.

MURPHY, G. I. 1965. A solution of the catch equation. J. Fish. Res. Board Can. 22(1): 191–202.

PALOHEIMO, J. E. 1980. Estimation of mortality rates. Trans. Am. Fish. Soc. 109: 378–386.

PAULY, D. 1980. On the interrelationships between natural mortality, growth parameters and mean environmental temperature in 175 fish stocks. J. Cons. Int. Explor. Mer 36: 175–192.

POPE, J. G. 1972. An investigation of the accuracy of virtual population analysis using cohort analysis. Int. Comm. Northw. Atlant. Fish. Res. Bull. 9: 65–74.

POPE, J. G., AND D. GRAY. 1983. An investigation of the relationship between the precision of assessment data and the precision of total allowable catches, p. 151–157. *In* W. G. Doubleday and D. Rivard [ed.] Sampling commercial catches of marine fish and invertebrates. Can. Spec. Publ. Fish. Aquat. Sci. 66.

POPE, J. G., AND J. G. SHEPHERD. 1985. A comparison of the performance of various methods for tuning VPAs using effort data. J. Cons. Int. Explor. Mer 42: 129–151.

PRAGER, M. H., AND A. D. MACCALL. 1988. Sensitivities and variances of virtual population analysis as applied to the mackerel, *Scomber japonicus*. Can. J. Fish. Aquat. Sci. 45: 539–547.

RICKER, W. E. 1973. Two mechanisms that make it possible to maintain peak-period yields from stocks of Pacific salmon and other fishes. J. Fish. Res. Board Can. 30: 1275–1286.

1975. Computation and interpretation of biological statistics of fish populations. Fish. Res. Board Can. Bull. 191: 382 p.

RIVARD, D. 1983. Effects of systematic, analytical, and sampling errors on catch estimates: a sensitivity analysis, p. 114–129. *In* W. G. Doubleday and D. Rivard [ed.] Sampling commercial catches of marine fish and invertebrates. Can. Spec. Publ. Fish. Aquat. Sci. 66.

RIVARD, D., AND M. G. FOY. 1987. An analysis of errors in catch projections for Canadian Atlantic fish stocks. Can. J. Fish. Aquat. Sci. 44: 967–981.

ROFF, D. A. 1984. The evolution of life history parameters in teleosts. Can. J. Fish. Aquat. Sci. 41: 989–1000.

ROTHSCHILD, B. J. 1986. Dynamics of marine fish populations. Harvard University Press, Boston, 277 p.

SAMPSON, D. B. 1987. Variance estimators for virtual population analysis. J. Cons. Int. Explor. Mer 43: 149–158.

1988. The stability of virtual population analysis cohort size estimates. J. Cons. Int. Explor. Mer 44: 135–142.

SAVILLE, A., AND R. S. BAILEY. 1980. The assessment and management of the herring stocks in the North Sea and to the west of Scotland. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 177: 112–142.

SHEPHERD, J. G., J. G. POPE, AND R. D. COUSENS. 1984. Variations in fish stocks and hypotheses concerning their links with climate. Rapp. P.-V. Reun. Cons. Int. Explor. Mer 185: 255–267.

SIMS, S. E. 1984. An analysis of the effect of errors in the natural mortality rate on stock-size estimates using virtual population analysis (cohort analysis). J. Cons. Int. Explor. Mer 41: 149–153.

ULLTANG, O. 1976. Catch per unit of effort in the Norwegian purse seine fishery for Atlanto–Scandinavian herring. FAO Fish. Tech. Pap. 155: 91–101.

1977. Sources of errors in and limitations of virtual population analysis (cohort analysis). J. Cons. Int. Explor. Mer 37: 249–260.

VETTER, E. F. 1988. Estimation of natural mortality in fish stocks: a review. Fish. Bull. 86: 25–42.

WAKABAYASHI, K. 1984. Estimations of biomass and yield for yellowfin sole in the eastern Bering Sea. Int. North. Pac. Fish. Comm. 42: 65–72.

WINTERS, G. M., E. L. DALLEY, AND J. A. MOORES. 1985. Fortuity disguised as fisheries management: the case history of Fortune Bay herring. Can. J. Fish. Aquat. Sci. 42 (Suppl. 1): 263–274.

Thyroid Hormone Content of Eggs and Early Developmental Stages of Five *Oncorhynchus* Species

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Measurements were made of thyroxine (T4) and triiodothyronine (T3) levels in the embryos and larvae of five species of the genus *Oncorhynchus*. Pink salmon (*O. gorbuscha*) embryos and larvae contained relatively low levels of both T4 and T3 throughout the early developmental stages. Sockeye salmon (*O. nerka*) had the highest levels of T4, with coho (*O. kisutch*), chum (*O. keta*), and chinook salmon (*O. tshawytscha*) having similar total T4 content. The chinook salmon embryos contained the highest T3 content of the five species studied, pink, sockeye, and chum salmon embryos had relatively low levels, and coho salmon embryos had intermediate levels of T3. In the pink salmon, there was little evidence of significant change in either T4 or T3 during development. In the other four species, the T4 content changed little between fertilization and hatch, but fell thereafter to stabilize at levels close to the lower detectable range of the assay used, except in the sockeye salmon larvae in which the values were considerably higher. The T3 content of chinook and coho salmon changed with development in a manner comparable with that observed for T4, but there was little evidence of a comparable posthatch decline in T3 levels in chum, sockeye, or pink salmon.

On a mesuré les concentrations de thyroxine (T4) et de triiodo-3,5,3′ thyronine (T3) dans les embryons et les larves de cinq espèces du genre *Oncorhynchus*. Les embryons et les larves de Saumon rose (*O. gorbuscha*) étaient relativement pauvres en T4 et en T3 aux premiers stades de leur développement. C'est chez le Saumon rouge (*O. nerka*) qu'on a mesuré la plus forte quantité de T4, les saumons coho (*O. kisutch*), kéta (*O. keta*) et quinnat (*O. tshawytscha*) ayant quant à eux une teneur totale en T4 similaire. Pour ce qui est de T3, ce sont les embryons de Saumon quinnat qui étaient les plus riches parmi les cinq espèces considérées, ceux des saumons rose, rouge et kéta se distinguant par des concentrations relativement faibles et ceux du Saumon coho par des concentrations moyennes. On a observé peu d'indices d'une variation significative de la teneur en T4 ou en T3 pendant le développement chez le Saumon rose. Chez les quatre autres espèces, les quantités de T4 ont peu changé entre la fertilisation et l'éclosion, mais ont baissé par la suite avant de se stabiliser à des niveaux proches de la quantité minimale détectable propre à la méthode d'analyse employée, sauf dans les larves de Saumon rouge, où les valeurs étaient considérablement plus élevées. Les concentrations de T3 ont changé au fur et à mesure du développement de façon comparable à celles de T4 chez les saumons quinnat et coho, mais peu d'indices permettaient de conclure à une diminution comparable, après l'éclosion, des quantités de T3 chez les saumons kéta, rouge et rose.

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Several recent studies have demonstrated and confirmed the presence of substantial amounts of the thyroid hormones thyroxine (T4) and triiodo-L-thyronine (T3) in the yolk of salmonid eggs and in the yolk contained within the yolk sac of the developing larval stages (Kobuke et al. 1987; Tagawa and Hirano 1987; Greenblatt et al. 1989). Work in salmonid fish and other species suggests that the thyroid hormones are of maternal origin (Brown et al. 1987, 1989); however, in the late stages of development, there is some evidence, at least in chinook (*Oncorhynchus tshawytscha*) and coho salmon (*O. kisutch*), for the contribution of the thyroid hormones of embryonic origin to the total hormone content of the embryo (Greenblatt et al. 1989).

These observations of relatively high levels of thyroid hormone in the yolk sac of salmonid fishes may be significant as regards the regulation of embryonic development, since the administration of T4 and T3 is known to alter certain developmental processes of fish, including the rate of absorption of the yolk from the yolk sac of some species (Dales and Hoar 1954; Lam 1980; Nacario 1983; Lam and Sharma 1985; Inui and Niwa 1985; Miwa and Inui 1987). Moreover, there is now evidence to indicate that by elevating the T3 content of eggs (by administering T3 to preovulatory females) it is possible to significantly increase the survival rates of the offspring (Brown et al. 1989). This suggests that the concentration of T3 (and/or T4) in the yolk is critical for optimal promotion of the early developmental processes, although in studies of thyroid hormone levels in embryos and larvae of Great Lakes coho salmon stocks, there was no correlation between thyroid hormone content and mortality (Leatherland et al. 1989).

This study compares the changes in levels of T4 and T3 in embryos and larvae of five species of oncorhynchid fish, coho, chinook, chum (*O. keta*), pink (*O. gorbuscha*), and sockeye salmon (*O. nerka*) collected from various stocks in British Columbia, to determine whether differences (if any) in egg thyroid hormone content are related to different life history strategies in the five species. In addition, the thyroid hormone levels were measured in three separate stocks of coho salmon to determine whether the egg thyroid hormone content varies among different stocks of the same species.

Materials and Methods

Source and Collection of Gametes, and Rearing Conditions for Eggs and Larvae

Gametes were collected from salmon at several Department of Fisheries and Oceans salmonid propagation facilities in British Columbia and were reared at either the West Vancouver Laboratory or the Cultus Lake Laboratory. The origin of the stocks used, the parental crosses used, the rearing sites, and estimates of survival to appearance of eye pigment are shown in Table 1. Coho salmon were collected from three separate locations (stocks), and four other Pacific salmon species, chinook, chum, pink, and sockeye salmon, were each represented by a single collection of gametes. The gametes were transported on ice to their respective rearing facility; eggs from several females were pooled and fertilized with milt pooled from several males (see Table 1 for the date and number of parents used for each cross). The fertilized eggs were water hardened, surface disinfected with a solution of iodine as described by Morrison et al. (1985), and placed in "Heath Stack" incubators (Heath Techna Corp., Kent, WA). When the majority of embryos of each species or stock exhibited eye pigment, the tray containing the eggs was agitated to provide a mild physical shock to identify unfertilized eggs which became opaque within minutes of applying such treatment. Dead eggs were removed and counted during routine sampling in order to estimate survival rates; antifungal treatments were not used.

Temperature of the well water supplying the West Vancouver Laboratory incubators was recorded daily and was relatively constant at 10.4 ± 0.4°C (mean ± SD). The temperature of the spring water supplying the Cultus Lake Laboratory was recorded at irregular intervals but showed a small decline from a high of 10.5°C in October 1986 to a low of 8.7°C in January 1987. However, the mean rearing temperature of 9.4 ± 0.6°C was similar to that observed at West Vancouver.

Whole embryos were taken from each stock at 3- to 7-d intervals and frozen for thyroid hormone analysis.

Thyroid Hormone Analysis

The frozen whole eggs or larvae were transported on dry ice to the University of Guelph for measurement of total thyroid hormone content. The eggs and larvae were freeze-dried, weighed on a microbalance, minced where necessary, and then homogenized with 95% methanol containing 1 mmol of propylthiouracil (PTU) according to the methods described by Kobuke et al. (1987), Tagawa and Hirano (1987), and Greenblatt et al. (1989) using a Polytron homogenizer. The tissues were extracted twice with the methanol–PTU mixture, followed by sonication, vigorous vortexing for several minutes, and centrifugation at 3000 rpm for 10 min. The supernatants were vacuum-dried, reconstituted in barbitol buffer (pH 8.6) containing 2 mg anilinonaphthalene sulfonic acid (ANS)·mL^{-1} according to the method of of Greenblatt et al. (1989) to increase the efficiency of extraction of the thyroid hormones, and reextracted with an ethanol–chloroform mix (1:2 v/v) to remove lipids. The thyroid hormones contained in the aqueous layer were assayed using radioimmunoassays (RIA) (Jolden Diagnostics, Scarborough, Ont.), the characteristics of which have been described previously (Burke and Leatherland 1983). The extraction efficiency was >75% when estimated by adding either radiolabelled L-thyroxine (T4) to the sample at the time of the first homogenization and determining the recovery of the label at the final stage or by adding known amounts of unlabelled T4 to homogenates of eggs or larvae and measuring the T4 appearing at the final stage by means of RIA. The data presented have not been corrected for extraction efficiency.

Statistical Analysis

The data of T4 and T3 content of the embryos and larvae were separately subjected to one-way analysis of variance. Sample sizes of 10 organisms were used throughout for both T4 and T3 measurements (a total of 20 eggs·sample time^{-1}). Where *F* values indicated significance, differences between means were compared using Tukey's honestly significant difference test according to the procedure of Steel and Torrie (1980). The 95% confidence level ($p < 0.05$) was used unless otherwise stated. The data were log-transformed before statis-

TABLE 1. Sources and rearing information for *Oncorhynchus* species used in the study.

Species	Origin[a]	Parental cross[b]	Date of fertilization	Rearing site[c]	Survival (%)[d]	Appearance of eye pigment[e]	Hatch[e]	Yolk absorption and first feed[e]
Chinook	Big Qualicum	3 × 3	Nov. 5, 1986	WV	40	26/270	54/561	100/1044
Chum	Chilliwack	5 × 5	Nov. 19, 1986	WV	60	26/269	55/575	93/966
Coho	Big Qualicum	5 × 6	Nov. 11, 1986	WV	>90	21/217	46/481	77/804
Coho	Chilliwack	5 × 5	Oct. 27, 1986	CL	NA	26/247	52/461	92/826
Coho	Capilano	6 × 6	Nov. 13, 1986	WV	>90	22/228	48/500	78/814
Pink	Quinsam	12 × 10	Oct. 9, 1986	WV	>90	26/277	64/671	106/>1123
Sockeye	Weaver Creek	7 × 7	Oct. 27, 1986	CL	NA	26/247	52/461	92/826

[a] Hatchery or river.

[b] No. of males × no. of females used in the cross.

[c] WV, West Vancouver; CL, Cultus Lake.

[d] Estimated % of embryos at eye pigment appearance stage.

[e] Days after fertilization/accumulated thermal units (no. of days × °C·d^{-1}).

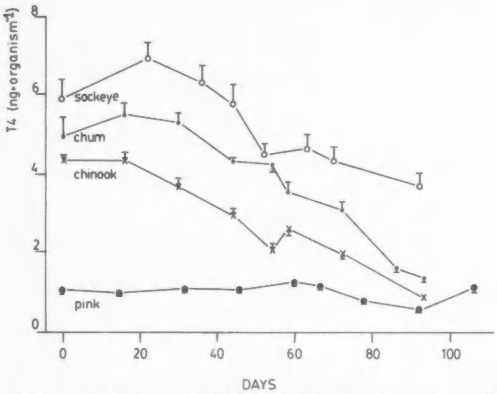

FIG. 1. Changes in thyroxine (T4) content (mean ± SEM, $n = 10$) of sockeye, chum, chinook, and pink salmon embryos and larvae sampled at different developmental stages.

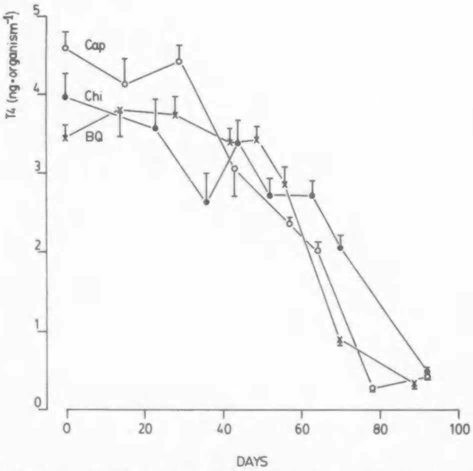

FIG. 2. Changes in thyroxine (T4) content (mean ± SEM, $n = 10$) of three stocks (Capilano Hatchery (Cap), Chilliwack Hatchery (Chi), Big Qualicum Hatchery (BQ)) of coho salmon embryos and larvae sampled at different developmental stages.

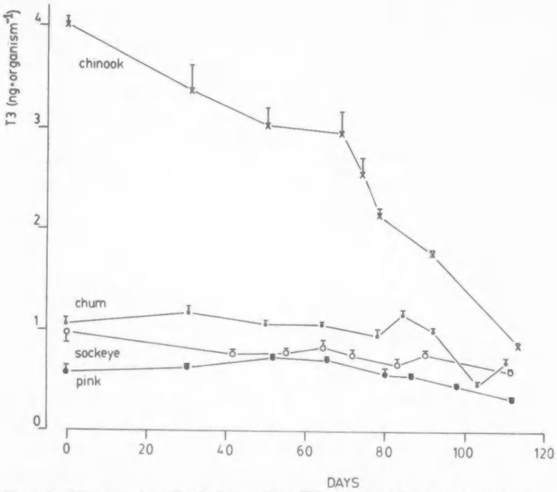

FIG. 3. Changes in triiodothyronine (T3) content (mean ± SEM, $n = 10$) of chinook, chum, sockeye, and pink salmon embryos and larvae sampled at different developmental stages.

tical analysis to satisfy the criteria of homogeneity of variance between treatment groups.

Results

Egg and Larval Content of L-Thyroxine (T4)

Unfertilized eggs (day 0)

The mean T4 content of the eggs of pink salmon was significantly lower ($p < 0.01$) than in all other stocks examined in the study (Fig. 1). The egg T4 content of sockeye, chum, and chinook salmon were not significantly different from one another. There were no significant differences in egg T4 content of the three stocks of coho salmon (Fig. 2). The T4 content in the Big Qualicum (BQ) coho salmon eggs was significantly lower ($p < 0.01$) than that of the sockeye salmon.

Eye-pigment appearance stage (21–26 d)

The T4 content of the embryos of pink salmon sampled just after the appearance of eye pigment was significantly lower ($p < 0.01$) than in any of the other groups (Fig. 1). In addition, the T4 content of the sockeye eggs was significantly greater ($p < 0.01$) than in two of the three stocks of coho salmon (BQ and Chilliwack (Chi)) and those of the chinook salmon. There were no differences between the three stocks of coho salmon (Fig. 2) or between sockeye salmon and chum salmon eggs.

From fertilization (day 0) to late embryonic (prehatch) stage (46–64 d)

Except for a significant ($p < 0.05$) lowering of T4 content of the eggs of the Capilano (Cap) coho salmon stocks between fertilization and the prehatch period, there were no other significant changes in egg T4 content during the same period in any of the other two stocks of coho salmon or four other salmon species examined.

Eighty-nine to 92 d postfertilization (time of first exogenous feeding)

The sockeye salmon larvae contained significantly ($p < 0.01$) more T4 than any of the other species examined at 89–92 d postfertilization. In addition, whole-body T4 content of the larvae of coho and pink salmon was significantly lower than the T4 content of the chinook and chum salmon ($p < 0.01$), except for the comparison of pink and chinook salmon where $p < 0.05$).

Egg and Larval Content of Triiodo-L-Thyronine (T3) (Fig. 3 and 4)

Unfertilized eggs (day 0)

The T3 content of the unfertilized eggs of chinook salmon was three- to fourfold higher than in any other group examined ($p < 0.01$), and that of pink salmon eggs was significantly lower than all other groups ($p < 0.01$, except for comparison with sockeye salmon where $p < 0.05$, Fig. 3). In addition, T3 levels in the Chi and BQ coho salmon eggs were significantly higher than those of sockeye, pink, chinook ($p < 0.01$) and chum salmon eggs ($p < 0.01$) (Fig. 4).

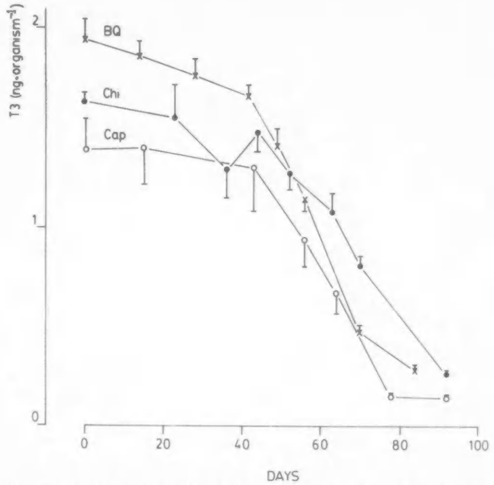

FIG. 4. Changes in triiodothyronine (T3) content (mean ± SEM, $n = 10$) of three stocks (Big Qualicum (BQ), Chilliwack (Chi), Capilano (Cap)) of coho salmon embryos and larvae sampled at different development stages.

Eye pigment appearance stage (21–26 d)

Total T3 content of the chinook salmon embryos sampled just after the appearance of eye pigment was significantly higher ($p < 0.01$) than in all other groups (Fig. 3). There were no significant differences between the three stocks of coho salmon (Fig. 4). However, the T3 content of the Cap and BQ coho salmon embryos was significantly higher ($p < 0.01$) than that of the chum, sockeye, and pink salmon embryos. Additionally, the T3 levels in the chum and Chi coho salmon were higher than in the sockeye ($p < 0.05$) and pink salmon ($p < 0.01$).

From fertilization (day 0) to late embryonic (prehatch) stage (46–64 d)

There was no significant change in the egg content of T3 between fertilization and immediately prehatch in any of the species examined.

Eighty-nine to 92 d postfertilization (time of first exogenous feeding)

Total T3 content of the chinook salmon larvae was significantly higher ($p < 0.01$) than in all other groups, and levels in sockeye salmon larvae were significantly higher than in the three stocks of coho ($p < 0.01$) and pink salmon ($p < 0.05$).

Discussion

The results of the present study confirm the presence of significant amounts of both T4 and T3 in the eggs (embryos) of coho, chinook, and chum salmon as reported earlier by others (Kobuke et al. 1987; Tagawa and Hirano 1987; Greenblatt et al. 1989) and show a similar occurrence in the eggs (embryos) of sockeye and pink salmon. Moreover, the study shows that in addition to marked interspecies differences in the levels of total thyroid hormones, the relative levels of the two hormones (T4 and T3) in the different species also vary markedly.

Throughout the paper, thyroid hormone levels are expressed as nanograms per organism. Moreover, because there are significant differences between species and between different stocks of the same species as regards the size of eggs and larvae, the tissue concentrations of the hormones, rather than the total hormone content of each organism, might be more relevant. For example, the highest total T4 content was evident in sockeye salmon (Fig. 1) which have the smallest eggs and larvae (Table 2); thus, in this species the concentration of hormone within the tissues is considerably higher than in the other species examined. Conversely, whereas the total T4 content of pink salmon embryos was lower than that of any of the other species, the T4 content per unit weight was of the same order as that of chinook salmon.

TABLE 2. Dry weight (mg, mean ± SEM, $n = 10$) of eggs and larvae of coho, chinook, chum, sockeye, and pink salmon at three stages of development. BQ, Big Qualicum Hatchery; Chi, Chilliwack Hatchery; Cap, Capilano Hatchery.

Species	Stage of development: Day of fertilization	Eye pigment appearance	89–92 d postfertilization
Chinook	195.5 ± 2.2^a	177.3 ± 6.9^a	131.9 ± 3.5^a
Chum	106.1 ± 4.8^b	122.9 ± 1.2^b	82.1 ± 3.2^b
Coho BQ	87.0 ± 3.0^c	90.5 ± 3.8^c	59.4 ± 3.9^e
Chi	74.2 ± 2.3^d	63.8 ± 4.6	43.7 ± 2.0
Cap	73.8 ± 5.4	65.7 ± 4.0	48.3 ± 4.8
Pink	65.2 ± 2.1	71.7 ± 1.4	51.1 ± 1.2
Sockeye	59.0 ± 1.0	59.3 ± 1.5	44.1 ± 0.9

[a] Significantly larger than all other groups.
[b] Significantly larger than coho, pink and sockeye salmon.
[c] Significantly larger than Chi and Cap coho, pink, and sockeye salmon.
[d] Significantly larger than sockeye salmon.
[e] Significantly larger than Chi coho and sockeye salmon.

Of the five species examined in this study the highest embryonic T3 content was found in the chinook salmon (approximately 4 ng·organism^{-1}) which was two- to fourfold higher than in any of the other species or stocks (Fig. 3 and 4), including the three stocks of coho salmon examined. This observation is in contrast with that of Greenblatt et al. (1989) who found similar T3 levels (approximately 5 – 7 ng·organism^{-1}) in coho and chinook eggs (embryos). We have no satisfactory explanation for the discrepancy between the two studies, although it is possible that there were differences between the different stocks used. When the T3 content data were expressed as nanograms per unit dry weight of embryo however, the levels for chinook and coho salmon were similar (20–25 ng·g^{-1}).

For sockeye salmon, despite their high embryonic T4 content (Fig. 1), the T3 content was relatively low, comparable with that of pink and chum salmon (Fig. 3). However, when these values are prorated to unit dry weight of sample, sockeye salmon have T3 levels that are similar to those of coho and chinook salmon.

In four of the five species examined, the pattern of T4 changes in the embryos and larvae was similar. The exception was the pink salmon which exhibited no apparent variation in T4 content over the 92 d of the study (Fig. 1). In the remaining species, however, there tended to be either no change in embryo T4 content between fertilization and hatch or a modest (albeit significant) decline, followed by a more marked fall in hormone content over the first 30 d posthatch to reach levels of approximately 1 ng·organism^{-1} (Fig. 1 and 2). This posthatch decline in T4 was not as pronounced in sockeye salmon, in which T4 levels even 90+ d after fertilization were still approximately 4 ng·organism^{-1} (Fig. 1). These changes in embryonic T4 content of coho, chinook, and chum salmon following hatch are in general agreement with reports by previous investigators (Kobuke et al. 1987; Tagawa and Hirano 1987; Greenblatt et al. 1989), although the extent of the changes reported here is more consistent with Kobuke et al.'s (1987) and Tagawa and Hirano's (1987) findings than with those of Greenblatt et al. (1989). Moreover, 20- to 25-fold increase in embryonic T4 content reported by Greenblatt et al. (1989) in chinook salmon 24 d after fertilization was not evident in the study reported here.

When the data are expressed as nanograms per unit dry weight, thus removing the confounding factor of increasing water content of the embryos during development, the patterns of changing hormone levels differ from those based on nanograms per organism data. A marked decline in T4 content per unit dry weight was most evident in coho and chum salmon; the values fell from approximately 40–50 ng·g dry weight^{-1} in the unfertilized eggs of all three stocks to values of 7–12 and approximately 20 ng·g dry weight^{-1} for coho and chum salmon, respectively, 82–94 d after fertilization which represent >75, >90, and >61% decreases for Chi coho, BQ coho, and chum salmon, respectively. During the same period, the decrease in absolute T4 levels expressed as nanograms per unit dry weight of chinook and sockeye salmon larvae was not as marked, although the percent fall in the chinook salmon (70%) was comparable with that of the coho and chum; that of the sockeye (34%) was the lowest of the five species examined. However, whereas in the chinook salmon the final T4 content was <10 ng·g dry weight^{-1}, that of the sockeye salmon stabilized at >70 ng·g dry weight^{-1}.

The total embryonic T3 content of chinook and coho salmon decreased by >75 and >85%, respectively, from day 0 to 92 postfertilization (Fig. 3 and 4); similar changes are evident if the data are expressed as nanograms per gram dry weight. No such change was evident in chum, sockeye, and pink salmon in which the embryonic T3 content was low (approximately 1 ng·organism^{-1}) throughout the study period (Fig. 3). These major changes in T3 content of the chinook and coho salmon embryos were not evident in the report of Greenblatt et al. (1989) in which the levels were found to remain in the 8–20 ng·organism^{-1} range throughout development. Since the extraction protocol, extraction efficiency, T3 RIA sensitivity, and reported embryo T4 content were essentially similar in this study and in that of Greenblatt et al. (1989), we have no satisfactory explanation for the apparent discrepancies between the two studies.

Greenblatt et al. (1989) provided evidence for the commencement of thyroid hormone production by the embryos of both coho and chinook salmon approximately 25–30 d after fertilization. The evidence is based on the appearance of sustained iodide uptake activity, which coincided with the >10-fold rise in total T4 content of chinook, but not coho embryos. We have no evidence from the study reported here, for or against, such a commencement of endogenous thyroid activity. However, as discussed above, the sharp rise in total T4 content of the chinook embryos between days 24 and 35 postfertilization (Greenblatt et al. 1989) was not found. In an earlier histological study of coho salmon the pituitary–thyroid axis had every appearance of being already functional in embryos up to 1 wk prior to hatch (Leatherland and Lin 1975). However, given the size of the thyroid tissue at that stage of development, it is difficult to conceive of the gland giving rise to a 10-fold increase in T4 content of the embryo, although such a possibility cannot be discounted.

Some of the differences between stocks might be related to the differences in conditions at the Cultus Lake and West Vancouver hatchery facilities, particularly differences in water temperature (see Materials and Methods). However, the similar patterns of hormone change in the three coho salmon stocks (two (Cap and BQ) raised at West Vancouver and the Chi raised at Cultus Lake) indicate that differences between hatcheries were not ipso facto the cause of the interspecies differences seen.

It is still not known how and at which stage of ovarian development the thyroid hormones enter the eggs, although based on the work of Brown et al. (1989), who showed that the egg T3 content could be elevated by administration of the hormone to the female 2 d before ovulation, it appears that addition of the hormone to the eggs can occur late in the ovarian developmental process. Some authors have suggested that the hormone is carried into the egg bound to vitellogenin, during vitellogenin incorporation into the gametes. This may be true in some species; for example, MacKenzie et al. (1987) found thyroid hormone binding to the relatively small vitellogenin molecules of channel catfish (*Ictalurus punctatus*), and Dickhoff and colleagues (cited in Greenblatt et al. 1989) found similar binding to vitellogenin of coho salmon. However, this may not be universal. For example, in estradiol-treated rainbow trout (*Oncorhynchus mykiss*) (formerly *Salmo gairdneri*), there was little or no binding of thyroid hormone to estradiol-induced vitellogenin, and in fact, there may have been a reduction in the total thyroid hormone binding activity, since the prealbumin-like fractions, which are the major thyroid hormone binding proteins in the species, were lower in the estradiol-treated animals (Flett and Leatherland 1989). Moreover, since T3 uptake by the gametes can occur just prior to ovulation (Brown et al. 1989), at a time when vitellogenin incorporation is presumably low, it is unlikely that thyroid hormone incorporation at that stage of gamete maturation is vitellogenin dependent.

It is tempting to link the rapid decline of blood thyroid hormone levels during the late sexual maturational stages of coho (Leatherland and Sonstegard 1980, 1981, 1987; Sower and Schreck 1982), sockeye (Biddiscombe and Idler 1983), chum (Ueda et al. 1984), and pink salmon (Leatherland et al. 1989) with the incorporation of thyroid hormones into the eggs. The decline may be due in part to such a process, since the decline in thyroid hormone levels in female coho and pink salmon is more rapid than that of the males (Leatherland and Sonstegard 1987; Leatherland et al. 1989). However, using coho salmon as an example, the total levels of thyroid hormone incorporated into gametes, calculated on the basis of a fecundity of approximately 2500–3000 eggs·female^{-1} (which is within the normal range for coho salmon), would be 5.0–6.0 and 11.3–13.5 g for T3 and T4, respectively. Since blood levels of T3 and T4 in maturing coho salmon are in the order of 1.5 and 4 g·dL^{-1}, respectively, there appears to be little necessity to lower the blood thyroid hormone levels in order to satisfy the requirements of the eggs. In fact, if the lowering of thyroid hormones is due to their removal from the circulation, one might expect a compensatory activation of the pituitary–thyroid axis and an additional secretion of T4. Since this does not occur, even in fish which have fully matured ova and which have retained their gametes well beyond the normal course (Leatherland and Sonstegard 1980, 1981), it is more likely that the reduction in thyroid hormone levels of the maturing adults is brought about by a change in the set point of the hypothalamic–pituitary–thyroid axis.

It is not clear whether these marked interspecies differences in thyroid hormone content are related to the biology of the different species. Pink salmon have the lowest thyroid hormone reserves, and chum salmon, also, tend to have low values. Neither of these two species exhibts a pronounced smoltification process. However, with chinook, coho, and sockeye salmon there appears to be no such correlation between embryonic thyroid hormone content, period of residency in freshwater, or type of smoltification pattern. Thus, embryonic thyroid hormone titres per se may not be related closely to any particular aspect of developmental biology, but may simply reflect the thyroid hormone sequestering efficiency of the eggs of the different species. Certainly, for the coho salmon stocks examined, there is sufficient variability between stocks to suggest that variability between species may reflect normal biological variability. However, until there is a better understanding of the role of thyroid hormones in the developmental processes of salmonids, it is unwise to speculate excessively on the significance of interspecies variation.

One possible role for the high thyroid hormone levels in the embryos is that of metabolic regulation. Under conditions of reduced growth of salmonid fish (e.g. induced by fasting), plasma thyroid hormone levels fall (Eales 1988) and metabolite reserves are mobilized and utilized for energy production. In the salmonid embryo, two processes must be maintained: (1) mobilization of metabolite reserves (from the yolk sac) and (2) growth of the embryo. Perhaps the high thyroid hormone levels are required to ensure the maintenance of the growth process in concert with the metabolite mobilization process.

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References

BIDDISCOMBE, S., AND D. R. IDLER. 1983. Plasma levels of thyroid hormones in sockeye salmon (*Oncorhynchus nerka*) decrease before spawning. Gen. Comp. Endocrinol. 52: 467–470.

BROWN, C. L., I. DOROSHOV, M. COCHRAN, AND H. A. BERN. 1989. Enhanced survival in striped bass fingerlings after maternal triiodothyronine treatment. Fish Physiol. Biochem. 7: 295–299.

BROWN, C. L., C. V. SULLIVAN, H. A. BERN, AND W. W. DICKHOFF. 1987. Occurrence of thyroid hormones in early developmental stages of teleost fish. Am. Fish. Soc. Symp. 2: 144–150.

BURKE, M. G., AND J. F. LEATHERLAND. 1983. Seasonal changes in serum thyroid hormone levels of the feral brown bullhead, *Ictalurus nebulosus* Lesueur. J. Fish Biol. 23: 585–593.

DALES, S., AND W. S. HOAR. 1954. Effects of thyroxine and thiourea on the early development of chum salmon (*Oncorhynchus keta*). Can. J. Zool. 32: 244–251.

EALES, J. G. 1988. The influence of nutritional state on thyroid function in various vertebrates. Am. Zool. 28: 351–362.

FLETT, P. A., AND J. F. LEATHERLAND. 1989. *In vitro* hepatic monodeiodination of L-thyroxine and the temporal effect of 17β-estradiol on deiodination in rainbow trout (*Salmo gairdneri* Richardson). Fish Physiol. Biochem. 6: 129–138.

GREENBLATT, M., C. L. BROWN, M. LEE, S. DAUDER, AND H. A. BERN. 1989. Changes in thyroid hormone levels in eggs and larvae and in iodide uptake by eggs of coho and chinook salmon, *Oncorhynchus kisutch* and *O. tshawytscha*. Fish Physiol. Biochem. 6: (In press)

INUI, Y., AND S. MIWA. 1985. Thyroid hormone induces metamorphosis of flounder larvae. Gen. Comp. Endocrinol. 60: 450–454.

KOBUKE, L., J. L. SPECKER, AND H. A. BERN. 1987. Thyroxine content of eggs and larvae of coho salmon, *Oncorhynchus kisutch*. J. Exp. Zool. 242: 89–94.

LAM, T. J. 1980. Thyroxine enhances larval development and survival in *Sarotherodon mossambicus* Ruppell. Aquaculture 21: 287–291.

LAM, T. J., AND R. SHARMA. 1985. Effects of salinity and thyroxine on larval survival, growth and development of the carp, *Cyprinus carpio*. Aquaculture 44: 201–212.

LEATHERLAND, J. F., N. E. DOWN, H. DYE, AND E. M. DONALDSON. 1989. Changes in plasma thyroid hormone levels in pink salmon (*Oncorhynchus gorbuscha*) during their spawning migration in the Fraser River (Canada). J. Fish Biol. 35: 199–205.

LEATHERLAND, J. F., AND L. LIN. 1985. Activity of the pituitary gland of embryo and larval stages of coho salmon, *Oncorhynchus kistuch*. Can. J. Zool. 53: 297–310.

LEATHERLAND, J. F., L. LIN, N. E. DOWN, AND E. M. DONALDSON. 1989. Thyroid hormone content of eggs and early developmental stages of three stocks of goitred coho salmon (*Oncorhynchus kisutch*) from the Great Lakes of North America, and a comparison with a stock from British Columbia. Can. J. Fish. Aquat. Sci. 46: (This issue)

LEATHERLAND, J. F., AND R. A. SONSTEGARD. 1980. Seasonal changes in thyroid hyperplasia, serum thyroid hormone and lipid concentrations and pituitary gland structure in Lake Ontario coho salmon (*Oncorhynchus kisutch* Walbaum) and a comparison with coho salmon from Lakes Michigan and Erie. J. Fish Biol. 16: 539–562.

1981. Thyroid dysfunction in Great Lakes coho salmon, *Oncorhynchus kisutch* (Walbaum): seasonal and interlake differences in serum T3 uptake and serum total and free T4 and T3 levels. J. Fish Dis. 4: 413–423.

1987. Comparative fecundity and egg survival in two stocks of goitred coho salmon (*Oncorhynchus kisutch* Walbaum) from Lake Erie. Can. J. Zool. 65: 2780–2785.

MACKENZIE, D. S., J. WARNER, AND P. THOMAS. 1987. Thyroid–reproductive relationships in the channel catfish, *Ictalurus punctatus*: evidence for estradiol-induced changes in plasma thyroid hormone binding, p. 205. *In* D. R. Idler, L. W. Crim, and J. M. Walsh [ed.] Reproductive physiology of fish. Memorial University Press, St. John's, Nfld.

MIWA, S., AND Y. INUI. 1987. Effects of various doses of thyroxine and triiodothyronine on metamorphosis of flounder (*Paralichthys olivaceus*). Gen. Comp. Endocrinol. 67: 356–363.

MORRISON, P. F., J. F. LEATHERLAND, AND R. A. SONSTEGARD. 1985. Comparative fecundity and egg survival of coho salmon (*Oncorhynchus kisutch* Walbaum) from Lakes Ontario, Erie and Michigan. Can. J. Zool. 63: 1096–1100.

NACARIO, J. F. 1983. The effect of thyroxine on the larvae and fry of *Sarotherodon niloticus* L. (*Tilapia nilotica*). Aquaculture 34: 73–83.

SOWER, S. A., AND C. B. SCHRECK. 1982. Steroid and thyroid hormones during sexual maturation of coho salmon (*Oncorhynchus kisutch*) in seawater or fresh water. Gen. Comp. Endocrinol. 47: 42–53.

STEEL, R. G. D., AND J. H. TORRIE. 1980. Principles and procedures of statistics. McGraw-Hill Co., New York, NY. 481 p.

TAGAWA, M., AND T. HIRANO. 1987. Presence of thyroxine in eggs and changes in its content during early development of chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 68: 129–135.

UEDA, H., O. HIROI, A. HARA, K. YAMAUCHI, AND Y. NAGAHAMA. 1984. Changes in serum concentrations of steroid hormones, thyroxine, and vitellogenin during spawning migration of the chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 53: 203–211.

Thyroid Hormone Content of Eggs and Early Developmental Stages of Three Stocks of Goitred Coho Salmon (*Oncorhynchus kisutch*) from the Great Lakes of North America, and a Comparison with a Stock from British Columbia

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The thyroxine (T4) and triiodothyronine (T3) concentrations in embryos and larvae of two stocks of markedly goitred Lake Erie coho salmon (*Oncorhynchus kisutch*) were lower than in comparable developmental stages of a mildly goitred Lake Michigan and a nongoitred British Columbia stock. There was no apparent correlation between fecundity, egg weight, embryo mortality rates, or developmental rates of the three Great Lakes stocks and the egg yolk reserves of thyroid hormone, indicating that the reserves may not ipso facto affect developmental success. The low thyroid hormone levels of the embryos of the two Lake Erie stocks were probably related to the dysfunction in hormone uptake from the maternal blood during the period of oocyte growth, and not to low maternal blood thyroid hormone levels per se.

Les concentrations de thyroxine (T4) et de triiodo-3,5,3′ thyronine (T3) dans les embryons et les larves de deux stocks de Saumons coho (*Oncorhynchus kisutch*) fortement goitreux du lac Érié étaient inférieures à celles mesurées dans un stock moyennement goitreux du lac Michigan et un stock exempt de goitre de la Colombie-Britannique rendus à un stade de développement comparable. Les auteurs n'ont observé aucune corrélation apparente entre la fécondité, le poids des oeufs, les taux de mortalité des embryons ou les taux de développement des trois stocks des Grands Lacs et les réserves de vitellus de l'hormone thyroïdienne, ce qui indique que ces réserves ne déterminent pas nécessairement ipso facto la réussite du développement. Les faibles niveaux d'hormones thyroïdiennes des embryons des deux stocks du lac Érié étaient probablement dus à un dérèglement de la prise d'hormones à partir du sang de la mère pendant la croissance de l'ovocyte plutôt qu'au faible niveau des hormones thyroïdiennes dans ce sang même.

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In recent years there has been a growing interest in the thyroid hormone content of the eggs and early developmental stages of teleost fish, with particular reference to the possible role of these hormones in the control of the development of the embryos and larvae (Brown et al. 1987, 1989; Kobuke et al. 1987; Sullivan et al. 1987; Tagawa and Hirano 1987; Greenblatt et al. 1989). The L-thyroxine (T4) and triiodo-L-thyronine (T3) contained within the egg yolk and present in the yolk sac of the embryos and larvae appear to be of maternal origin. The evidence for this is derived from studies which show that treatment of sexually mature females with thyroid hormone prior to ovulation can significantly elevate the hormone content of the egg, and in some instances greatly improve the survival of the developing young (Brown et al. 1989). Thus, at least in some teleosts, the maternal thyroid hormones present in the yolk may well be an important factor in the successful hatching of the embryos and the development of the larval stages prior to the onset of endogenous thyroid hormone production.

Coho salmon (*Oncorhynchus kisutch*) were introduced into the Great Lakes of North America, from the 1960's onwards, as part of the Great Lakes reclamation programme. The original stocks of coho salmon were obtained from Oregon and British Columbia, and subsequent restocking programmes have used fingerlings raised from eggs taken from sexually mature fish collected during their potamodromous migration from Lakes Erie, Michigan, and Ontario. In addition, some self-reproducing runs of salmon have become established in several of the lakes.

The Great Lakes coho, chinook (*O. tshawytscha*), and pink salmon (*O. gorbuscha*) suffer epizootics of thyroid neoplasms (goitres) which are evident as histological lesions in all fish examined to date. In some instances (up to 90% in Lake Erie stocks) the lesions take the form of overt swellings that are visible grossly at the base of the gill arches (Leatherland and Sonstegard 1980, 1981a, 1981b, 1984; Moccia et al. 1981; Noltie et al. 1988). The thyroid lesions cannot be explained on the basis of iodide deficiency alone. They appear to have an environmental etiology (see Leatherland and Sonstegard 1984 for a review) and are prevalent prior to the onset of sexual maturation. Moreover, in coho salmon, there are interlake differences in the concentration of thyroid hormone in the serum of the adults during gonadogenesis (Leatherland and Sonstegard 1981a, 1981b) which are correlated with the prevalence and degree of the thyroid lesions in the adults (Leatherland and Sonstegard 1980).

If the early developmental processes of the embryos and larvae are influenced by the amount of thyroid hormone sequestered from the female by the developing eggs, then it follows that thyroid dysfunction in the adult Great Lakes coho (and other) salmon may have deleterious affects on the survival and maturation of the early developmental stages, and may explain, in part, the poor survival of embryos and larvae of some Lake Erie stocks (Morrison et al. 1985; Leatherland and Sonstegard 1987). In this study we measured the thyroid hormone content of the eggs and developing young of coho salmon taken from three stocks of Great Lakes salmon and one stock of salmon from British Columbia (Capilano River Hatchery). The three Great Lakes stocks display a wide variation in the degree of thyroid perturbations and embryo mortality, and the British Columbia stock served as a non-goitred "control." The study was undertaken to determine whether egg thyroid hormone content is correlated with the degree of thyroid perturbation of the adults, and to determine whether there is a correlation between egg thyroid hormone content and the size, fecundity, and survival of eggs and larvae of the Great Lakes stocks of coho salmon.

Materials and Methods

Source of Eggs

The three Great Lakes stocks used for the study were the Platte River Hatchery, Lake Michigan, stock (LM), the Fairview Hatchery, Lake Erie, stock (FH), and the Fishers Creek, Lake Erie, stock (FC). The LM stock was chosen because it has been found to have a high egg survival rate and low embryonic and larval mortality rate (Morrison et al. 1985); eggs from this stock have been used extensively for restocking both Lake Erie and Lake Michigan. The FH and FC stocks are both resident in Lake Erie; for several years the FH stocks has exhibited low egg survival and high embryonic mortality (see Morrison et al. 1985) whereas the FC stock is a self-reproducing stock which appears to produce viable eggs with high survival and low embryonic mortality characteristics (Leatherland and Sonstegard 1987). All three stocks exhibit thyroid lesions, but whereas those of the LM stock are evident only by histological examination, the two Lake Erie stocks show high prevalences of overt thyroid lesions (goitres).

Gametes were collected from these three stocks of Great Lakes coho salmon as well as from the Capilano River Salmon Hatchery stock from North Vancouver, British Columbia (BC), which was used as a control for the study.

For the three stocks of Great Lakes fish, both weight (to the nearest 10 g) and gonad weight (to the nearest 1 g) were measured to determine gonadosomatic indices (GSI = gonad weight/(total body weight − gonad weight)). The thyroid goitre index of a representative pool of fish from each stock was determined using the 1 – 5 scale described previously (Leatherland et al. 1982): stage 1, no thyroid lesion evident from gross examination of the gill arch region; stage 2, thyroid hyperplasia evident as a reddening of the tissue underlying the epidermis of the base of the third and fourth gill arches; stage 3, severe thyroid hyperplasia as evidenced by a small (<1 cm) protrusion at the base of the third and fourth gill arches; stage 4, distinct nodular lesion (>1 cm) restricted to one or two gill arches (usually, but not always bilateral); stage 5, multinodular bilateral lesions usually evident at the base of all the gill arches.

To obtain an estimate of fecundity of the three Great Lakes stocks, the total ovarian weight was measured in 10–12 preovulatory or early postovulatory females collected from each stock; samples of 20–40 unfertilized, unhardened eggs from each female were fixed in 10% formalin for determination of egg weight. The fecundity was calculated from the formula gonadal weight/mean egg weight of the formalin-fixed eggs.

From each of the four stocks, unfertilized eggs were taken from 10–12 sexually mature and ovulated females, placed in screwcap plastic vials, and frozen in liquid nitrogen. For the two Lake Erie stocks and the BC stock, eggs from 6–10 sexually mature and ovulated females were fertilized with a pool of milt from at least five males. For the LM stock, gametes from a large egg and milt pool (>30 females and >30 males) were used in the study. The eggs from the three Great Lakes stocks were water hardened in river water at the site of collection before being transported to the University of Guelph for incubation; they were maintained at 6–8°C during transportation. The gametes from the BC stock were transported to the West Vancouver Laboratory of the Biological Sciences Branch, Department of Fisheries and Oceans, for fertilization and incubation. The fertilized eggs were disinfected for 2 min with iodine as described previously (Morrison et al. 1985).

The eggs of the Great Lakes stocks were placed in horizontal trough incubating trays in constantly running and aerated well water at 10–11°C. To determine mortality rates in the three Great Lakes stocks, on the day following fertilization and transportation, groups of approximately 300 eggs were randomly assigned (10 groups·stock^{-1}) to 30 compartments in two identical hatching trays. At the same time, samples of the water-hardened eggs were taken for determination of water content and egg dry weight. The remaining eggs from each stock were retained in identical noncompartmentalized horizontal trough incubating trays under identical conditions to those described above and were used as the source of eggs for further sampling. Dead eggs were removed from the compartmentalized trays each day and from the other trays every 2–3 d. Ten days after collection of the gametes the number of dead eggs removed from each compartment was recorded until the time of appearance of eye pigment. At that time the remaining eggs were removed from each compartment, preserved in 10% formalin, and subsequently counted to determine the percent mortality during the period between fertilization and eye pigment stage. The 10-d interval between gamete collection and the commencement of the recording of dead eggs was intended to exclude mortalities which may have been directly attributable to transportation and initial egg handling.

Approximately 300 "eyed" eggs were then taken from each pool of the three Great Lakes stocks and randomly assigned to each of the 10 compartments for each stock, and the same procedure as that described above was used to measure the percent mortality during the period between eye pigment appearance and hatch.

After hatch the pools of fish from the three stocks were maintained in horizontal hatching trays until the yolk was absorbed, the swim bladder was filled, and the fish had begun to take exogenous food.

The fertilized eggs from the BC stock were incubated in a "Heath stack" incubator (Heath Techna Corp., Kent, WA) supplied with well water at a temperature of 10–11°C. When the majority of eggs had developed eye pigment, they were given a mild physical shock (agitation of the tray) to identify unfertilized eggs. Dead eggs were removed and counted during routine sampling in order to estimate survival rate.

Samples of 20–40 randomly chosen eggs or larval fish were taken at set times from each of the three Great Lake stocks, blotted dry, weighed, and frozen at −20°C until they were assayed for thyroid hormone content. Similar samples of the British Columbia stock were frozen at −30°C and shipped on dry ice to the University of Guelph for measurement of thyroid hormone content.

The extraction method used was that reported by Kobuke et al. (1987), Tagawa and Hirano (1987), and Greenblatt et al. (1989). Details of the procedure are reported elsewhere (Leatherland et al. 1989b).

The times after fertilization were calculated as accumulated thermal units (ATU = days × °C).

Data of body weight, GSI, and fecundity of the adults, weight of the eggs and larvae at different stages of development, thyroid hormone content of the eggs and larvae, and embryonic mortality (at the "eyed" stage and at hatch) were separately subjected to one-way analysis of variance. The thyroid hormone content data were log-transformed before statistical analysis to satisfy the requirements of homogeneity of variance. Weight data for the formalin fixed eggs of the three Great Lakes stocks (10 eggs taken from each of 10 females) were subjected to one-way analysis of variance with subsamples (Steel and Torrie 1980). Where *F* values indicated significance ($p < 0.05$), individual means were compared using Tukey's honestly significant different test. Data of thyroid goitre index were compared using Wilcoson's signed rank test using the Epistat software statistical package.

Results

Adult Condition

The body weight (total body weight minus gonad weight) of the females from the LM stock was significantly ($p < 0.01$) less than that of the males, but there were no sex differences in the FC and FH stocks. The body weights of the males and females of the LM stock were significantly smaller ($p < 0.01$) than males and females, respectively, of the FH and FC stocks (Table 1).

The GSI of the FH females was significantly ($p < 0.01$) larger than that of both the FC and LM stocks. The GSI of the males from all three stocks differed significantly from one another; that of the FH stock was the largest and that of the LM stock the smallest (Table 1).

There were no sex differences in goitre index in any of the three stocks. The index in the LM stock was significantly lower ($p < 0.01$) than in the two Lake Erie stocks for both males and females (Table 1).

There were no significant differences in the fecundity of the adult females of the three Great Lakes stocks (Table 1).

Egg Weight and Water Content

There were no significant differences between groups as regards the mean weights of the formalin-fixed unfertilized eggs or the mean weights of the fertilized eggs, but the mean dry weight of the eggs of the FC stock was significantly ($p < 0.01$) lower than that of the other two stocks (Table 2).

Despite the difference in dry weights, there were no differences in percent water content of the fertilized eggs.

Embryo Wet Body Weight and Body Water Content

There was no significant difference in the body weight of newly hatched alevins from the LM and FC stocks, but the newly hatched alevins of the FH stock were significantly smaller ($p < 0.01$) than those of the other two stocks (Table 2).

Approximately halfway through yolk reabsorption (70–72 d) the mean body weights of all three groups differed significantly ($p < 0.01$) from one another, with the FC stock being the largest and the FH stock the smallest (Table 2).

During the latter stages of yolk reabsorption (84–92 d), the mean body weights of all three groups differed significantly from one another ($p < 0.01$ for LM versus FC and FH; $p <$

TABLE 1. Body weight, gonadosomatic index (GSI), goitre index, and fecundity of sexually mature adult coho salmon from the Platte River, Lake Michigan (LM), Fishers Creek, Lake Erie (FC), and Fairview Hatchery, Lake Erie (FH), stocks. Data are shown as mean ± SEM (*n*). Means followed by a different letter are significantly different from one another ($p < 0.05$). Body weight and GSI of the BC stock of the females used as a source of gametes were 1.91 ± 0.28 kg and 23.3 ± 3.3 (mean ± SEM ($n = 5$)), respectively.

	LM		FC		FH	
	Male	Female	Male	Female	Male	Female
Body weight[a]	2.19 ± 0.19^{a} (10)	1.65 ± 0.12^{b} (10)	2.63 ± 0.09^{c} (47)	2.51 ± 0.08^{c} (62)	2.28 ± 0.07^{c} (82)	2.41 ± 0.06^{c} (77)
GSI[b]	3.2 ± 0.5 (10)	25.8 ± 1.6^{d} (10)	5.0 ± 0.4^{b} (19)	22.3 ± 1.2^{d} (13)	6.4 ± 0.2^{c} (73)	34.5 ± 1.0^{e} (46)
Goitre index[c]	2.6 ± 0.1^{a} (71)	2.4 ± 0.1^{a} (141)	4.0 ± 0.1^{b} (54)	4.1 ± 0.1^{b} (68)	4.1 ± 0.1^{b} (200)	4.1 ± 0.1^{b} (112)
Fecundity[d]	—	2889 ± 260^{a} (10)	—	3664 ± 236^{a} (10)	—	3629 ± 273^{a} (12)

[a] Body weight (kg) = total weight − gonad weight.

[b] GSI = gonad weight/(body weight − gonad weight) × 100.

[d] See Materials and Methods for explanation.

[d] Fecundity = number of eggs·female^{-1}.

TABLE 2. Egg weight and water content, embryo weight and water content, and embryo mortality of coho salmon from the Platte River, Lake Michigan (LM), Fishers Creek, Lake Erie (FC), and Fairview Hatchery, Lake Erie (FH), stocks. Data are shown as mean ± SEM (number of samples or replicates); all weights are expressed in g; water contents and mortalities are expressed as %. Means followed by a different letter are significantly different from one another ($p < 0.05$, Tukey's W test). The mean weight of unfertilized formalin-fixed eggs of BC coho salmon (20-22 eggs from each of 12 females) was 0.157 ± 0.007, water content of fertilized and water hardened eggs was 69.4% and dry weight was 0.063 $g \cdot egg^{-1}$.

	LM	FC	FH
Egg weight (formalin fixed)[a]	0.147 ± 0.017^{a} (100)	0.186 ± 0.017^{a} (100)	0.169 ± 0.017^{a} (100)
Egg weight (wet)[b]	0.173 ± 0.007^{a} (10)	0.183 ± 0.008^{a} (10)	0.177 ± 0.005^{a} (10)
Egg weight (dry)[c]	0.076 ± 0.003^{a} (10)	0.054 ± 0.005^{b} (10)	0.078 ± 0.002^{a} (10)
Egg water content[d]	56.1 ± 0.4^{a} (10)	57.3 ± 0.4^{a} (10)	56.2 ± 0.4^{a} (10)
Embryo weight[e]			
425–435 ATU	0.169 ± 0.004^{a} (20)	0.185 ± 0.004^{a} (20)	0.146 ± 0.007^{b} (20)
620–650 ATU	0.236 ± 0.004^{a} (20)	0.259 ± 0.006^{b} (20)	0.174 ± 0.004^{c} (20)
710–740 ATU	0.397 ± 0.008^{a} (20)	0.338 ± 0.017^{b} (20)	0.283 ± 0.15^{c} (20)
Embryo water content[f]			
450–460 ATU	62.2 ± 0.2^{a} (10)	61.0 ± 0.2^{a} (10)	62.3 ± 0.2^{a} (10)
710–740 ATU	82.3 ± 0.3^{a} (10)	81.8 ± 0.2^{a} (10)	81.6 ± 0.4^{a} (10)
Embryo mortality[f]			
Period 1	8.2 ± 0.5^{a} (10)	7.0 ± 0.6^{a} (10)	19.0 ± 0.9^{b} (10)
Period 2	25.6 ± 1.7^{a} (10)	6.6 ± 0.4^{b} (10)	45.5 ± 1.1^{c} (10)
Time to eye pigment appearance[g]	21/209	20/207	28/245
Time to hatch[g]	46/428	47/433	48/425
Time to yolk absorption and filling of swim bladder[g]	92/792	95/820	97/809

[a] Formalin-fixed unfertilized eggs (10 eggs from each of 10 females).

[b] Fresh eggs weighed 1 d after fertilization and water hardening.

[c] Dry weight of eggs weighed for footnote b.

[d] Water content of eggs weighed for footnote b.

[e] Wet weight of embryos measured at hatch (426–435 accumulated thermal units (ATU)), approximately midway through yolk absorption (620–650 ATU) and toward the end of yolk absorption, but prior to filling of swimbladder (710–740 ATU).

[f] Embryo mortalities are shown for the period between 10 d posthatch to the appearance of the eye pigment (period 1) and the period between the appearance of eye pigment and hatch (period 2). The numbers in parentheses after the mean mortalities indicate replicates of approximately 300 $fish \cdot replicate^{-1}$.

[g] Days after fertilization/ATU.

0.05 for FC versus FH), but by this stage the LM stock was the largest and the FH stock the smallest.

The percent water content of the embryos of all three stocks of Great Lakes salmon rose progressively during development from values of about 62% in animals at the time of hatching to about 82% at the time of yolk reabsorption; there were no significant differences among the three stocks (Table 2).

Embryo Mortalities

The mortality of the BC stock between fertilization and eye pigment formation was assessed as less than 10%, but the data were not derived from a random block experiment.

Mortalities of the LM, BC, and FC stocks were similar in the first developmental period examined; the percent mortality of the FH stock was significantly ($p < 0.01$) higher than that of the LM and FC stocks. During the second developmental period examined (eye pigment formation to hatch) the mortalities (mean ± SEM) of all three Great Lakes stocks differed significantly ($p < 0.01$) from one another, with the FC stock having the lowest (6.6 ± 0.4), the FH stock the highest (45.5 ± 1.06), and the LM stock being intermediate (25.6 ± 1.67).

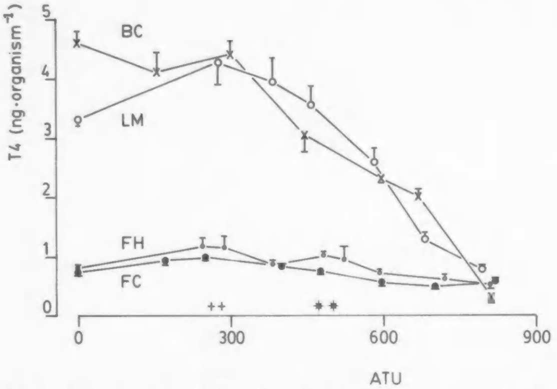

FIG. 1. Changes in thyroxine (T4) content (mean ±SEM, $n = 10$) of embryos and larvae of coho salmon taken at different stages of their early development from the Capilano Hatchery, British Columbia (BC), Platte River, Michigan (LM), Fairview Hatchery, Pennsylvania (FH), and Fishers Creek, Ontario (FC) stocks. Asterisks indicate the period of hatching; plus symbols indicate the period of eye pigment appearance. ATU = accumulated thermal units (number of days × °C).

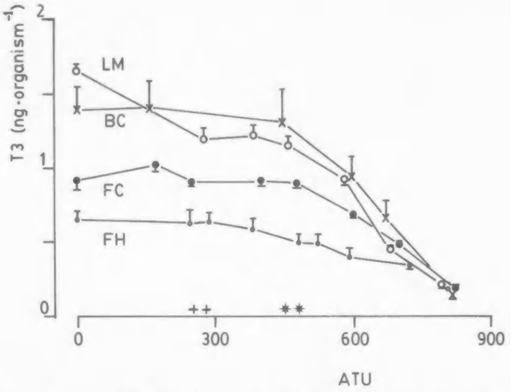

FIG. 2. Changes in triiodothyronine (T3) content of embryos and larvae of coho salmon taken at different stages of their early development from four stocks of coho salmon (see legend to Fig. 1).

Thyroid Hormone Content of Eggs and Early Developmental Stages

The total T4 content of the newly fertilized eggs of the BC stock was significantly higher ($p < 0.01$) than that of all three Great Lake stocks (Fig. 1). The T4 content of the newly fertilized eggs of the LM stock was significantly higher ($p < 0.01$) than that of both the FH and FC stocks; the T4 content of the latter two stocks was similar.

Between fertilization and hatching, the T4 content of the eggs (or embryos) of the three Great Lakes stocks did not change significantly, but there was a significant ($p < 0.01$) fall of T4 in the BC stock (Fig. 1). At hatching there was no significant difference between the T4 content of the larval fish from the LM or BC stocks, and both contained significantly higher ($p < 0.01$) levels than newly hatched fish of the FC and FH stocks (Fig. 1). By approximately 15 wk after fertilization (800 accumulated thermal units) there were no significant differences among the four stocks (Fig. 1).

There were no significant differences in total T3 content between newly fertilized eggs from the LM and BC stocks, but both contained significantly higher ($p < 0.01$) T3 levels than the eggs of the FC and FH stocks; the eggs of the FC stock contained significantly ($p = 0.05$) higher levels of T3 than those of the FH stock (Fig. 2).

Between fertilization and hatching there were no significant changes in the T3 content of the eggs and newly hatched larvae of the BC, FC, and FH stocks, but the LM stock exhibited a significant ($p < 0.01$) decrease (Fig. 2). The T3 content of the newly hatched larvae of the BC and LM stocks was similar. The T3 content of the newly hatched larvae of the FH stock was significantly ($p < 0.01$) lower than in the other three stocks, and that of the FC stock was significantly ($p < 0.05$) lower than that of the BC stock (Fig. 2). By approximately 15 wk after fertilization (800 accumulated thermal units) the T3 content of the larval fish of the four stocks was similar (Fig. 2).

Discussion

There is clear evidence of marked differences in the content of both T4 and T3 of embryos taken from three different stocks of coho salmon in the Great Lakes. The stock from the Platte River (LM) had significantly higher egg T4 and T3 levels than either of the Lake Erie stocks and at many sampling times was comparable with the BC stock. The two Lake Erie stocks had similar T4 content in the eggs and larval stages throughout development, but the T3 levels of the naturally spawning self-reproducing FC stock were significantly higher than those of the FH stock for much of the period studied.

These differences in thyroid hormone levels among the Great Lakes stocks were not due to differences in egg size, which were similar in all four stocks (Table 2).

Despite these marked differences in the thyroid hormone content of eggs from different stocks, there was no apparent relationship between the hormone content and embryonic or larval survival rates. The highest mortality (64.5%) was evident in the FH stock, but the lowest mortality was in the FC stock (13.6%) which had egg T4 levels which were similar to those of the FH stock. Moreover, although the egg T3 levels of the FC stock were higher than those of the FH stock, T3 levels were significantly lower than those of the LM stock which had a cumulative mortality of 33.8%. This suggests that the high mortality of the FH stock is not ipso facto related to the relatively low thyroid hormone levels in the eggs. Similarly, the relatively high thyroid hormone levels in the LM stock did not appear to increase the survival of the embryos and larvae of that stock. This finding is at odds with studies of Brown et al. (1989) who reported an increased survival and growth rate of striped bass (*Morone saxatilis*) embryos taken from females which had been injected with T3 2 d prior to removal of the eggs.

Similarly, in the salmon used in the present study, there appeared to be no marked difference in the incubation period of the eggs from the different stocks, or in the relative rate of yolk absorption (Table 2). This observation is significant given the considerable evidence showing that T4 or T3 enhances the rates of embryonic development and larval yolk absorption of several species of teleost fish, including chum salmon (*O. keta*) (Dales and Hoar 1954), tilapia (*Sarotherodon mossambicus*) (Lam 1980), carp (*Cyprinus carpio*) (Lam and Sharma 1985), milkfish (*Chanos chanos*) (Lam et al. 1985), flounder (*Paralichthys olivacens*) (Inui and Miwa 1985; Miwa and Inui 1987), and maternally T3-injected striped bass (Brown et al. 1989). A possible explanation for this apparent discrepancy is that the endogenous yolk-bound thyroid hormone does not have access to target sites in the embryo until it is released into the blood of the embryo or larva. The release is likely associated with the yolk absorption process. During this process, the animal may well have the capacity to regulate blood thyroid hormone levels by altering T4 and T3 excretion rates and possibly also T4 monodeiodination rates. Thus, embryonic and larval blood thyroid hormone levels may be regulated within required limits, and thus remain largely independent of the *amount* of the total thyroid hormone reserve of the yolk. In this way the embryos and larvae would be able to utilize the hormone reserve for whatever regulatory role is required, but would not be themselves "driven" metabolically by the size of the reserve, over which they have no control. Thus, the differences in *endogenous* total thyroid hormone levels of the eggs, embryos, and larvae need not necessarily reflect the level of hormone presented to the developing tissues. Conversely, where *exogenous* hormone is provided, the concentration of thyroid hormone presented to the tissues may be elevated, since there is no control process to prevent it, and thus an increase in the developmental processes will be manifested.

In the present study, it is possible that some of the differences in mortality are related to the different transportation distances from the site of collection and fertilization to the holding facility in Guelph (1-, 4-, and 10-h journeys for the FC, FH, and LM stocks, respectively). However, the mortality over the first 10 d was not included in the data reported here in order to compensate for such effects. Moreover, the mortality rates of the LM and FH stocks held in the Guelph facility were comparable with those reported by the Michigan and Pennsylvania fisheries agencies (C. Pecor (Michigan) and N. Shea (Pennsylvania), unpubl.).

The thyroid hormone content of the eggs of the LM stock was generally comparable with that of the BC coho salmon eggs, despite the presence of thyroid lesions in the LM adults. There are no data as to the thyroid hormone levels of the LM coho salmon during the period of egg formation, but presumably the blood hormone levels were sufficient to enable the incorporation of "normal" levels of hormone into the developing gametes. The situation in the Lake Erie fish is less clear. Plasma thyroid hormone levels in Lake Erie coho salmon collected in the spring when the gonad was enlarging prior to final gamete maturation were relatively high (>4 ng·mL^{-1} for T4 and >28 ng·mL^{-1} for T3) (Leatherland and Sonstegard 1987). Despite these remarkably high plasma hormone levels, there appears to be relatively little transfer of that hormone from the plasma into the egg. The mechanism by which thyroid hormones are transferred to the eggs is as yet unclear. Estrogen-induced vitellogenin does not appear to preferentially bind thyroid hormones in rainbow trout (*Salmo gairdneri*) (Flett and Leatherland 1989), although in channel catfish (*Ictalurus punctatus*), which may have a smaller vitellogenin molecule than salmonid fishes, there is evidence of thyroid hormone binding capacity (MacKenzie et al. 1987). It remains to be determined whether the apparent dysfunction in thyroid hormone transfer to the eggs in the FH stock is related to a parallel dysfunction of either vitellogenin synthesis and/or transfer.

It has been argued (Greenblatt et al. 1989) that the fall in blood thyroid hormone levels of Pacific salmon during the late period of gonadal maturation (and associated potamodromous or anadromous migration) (Leatherland and Sonstegard 1980, 1981b, 1987; Sower and Schreck 1982; Biddiscombe and Idler 1983; Ueda et al. 1984; Leatherland et al. 1989a), which occurs more rapidly in females than in males (Leatherland and Sonstegard 1987; Leatherland et al. 1989a), is brought about by the incorporation of T4 and T3 into the ovary. However, ovarian maturation in oncorhynchid species takes place when the plasma thyroid hormone levels are high, during late summer and early fall, and its is not until the eggs are ready for ovulation that the serum thyroid hormone levels fall (Leatherland and Sonstegard 1980). Moreover, the total amount of thyroid hormone accumulated by the ovary (7.2, 2.2, and 3.2 μg of T4 for the LM, FC, and FH stocks, respectively, and 3.6, 2.7, and 2.7 μg of T3 for the BC, FC, and FH stocks, respectively), even assuming that all the accumulated T4 and T3 in the ovary was acquired during the immediate prespawning period, could not account for the disappearance of these hormones from the blood. Indeed, if T4 was removed from the circulation by ovarian acquisition, one might expect a compensatory secretion of T4 by removal of the negative feedback control of the hypothalamic–pituitary–thyroid axis and a subsequent recovery of plasma thyroid hormone levels. This does not occur, even in spent females. Moreover, the fact that the decline in blood T4 and T3 levels occurs in the males of these species, also, suggests that it is the result of a down regulation of the hypothalamic–pituitary–thyroid axis taking place during this phase of the life cycle, perhaps permitting mobilization of energy resources necessary for migration and/or final maturation of the testes and ovaries. An additional argument against the theory of a significant ovarian involvement in the control of thyroid hormone reserves in the adults comes from studies of the prevalence of goitres in Great Lakes salmon which have been made over a period of 10–12 yr by the authors. If the ovary is a significant factor as regards thyroid hormone accumulation, one would expect to find a higher prevalence of lesions (and/or larger lesions) in females compared with males. No such epizootiological pattern has so far emerged from studies of the Lakes Michigan, Ontario, and Erie stocks (J. F. Leatherland and R. A. Sonstegard, unpubl. data).

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References

Biddiscombe, S., and D. R. Idler. 1983. Plasma levels of thyroid hormones in sockeye salmon (*Oncorhynchus nerka*) decrease before spawning. Gen. Comp. Endocrinol. 52: 467–470.

Brown, C. L., I. Doroshov, M. Cochran, and H. A. Bern. 1989. Enhanced survival in striped bass fingerlings after maternal triiodothyronine treatment. Fish Physiol. Biochem. 7: 295–299.

Brown, C. L., C. V. Sullivan, H. A. Bern, and W. W. Dickhoff. 1987. Occurrence of thyroid hormones in early developmental stages of teleost fish. Am. Fish. Soc. Symp. 2: 144–150.

Dales, S., and W. S. Hoar. 1954. Effects of thyroxine and thiourea on the early development of chum salmon (*Oncorhynchus keta*). Can. J. Zool. 32: 244–251.

Flett, P. A., and J. F. Leatherland. 1989. *In vitro* hepatic monodeiodination of L-thyroxine and the temporal effect of 17β-estradiol on deiodination in rainbow trout (*Salmo gairdneri* Richardson). Fish Physiol. Biochem. 6: 129–138.

GREENBLATT, M., C. L. BROWN, M. LEE, S. DAUDER, AND H. A. BERN. 1989. Changes in thyroid hormone levels in eggs and larvae and in iodide uptake by eggs of coho and chinook salmon, *Oncorhynchus kisutch* and *O. tshawytscha*. Fish Physiol. Biochem. 6: (In press)

INUI, Y., AND S. MIWA. 1985. Thyroid hormone induces metamorphosis of flounder larvae. Gen. Comp. Endocrinol. 60: 450–454.

KOBUKE, L., J. L. SPECKER, AND H. A. BERN. 1987. Thyroxine content of eggs and larvae of coho salmon, *Oncorhynchus kisutch*. J. Exp. Zool. 242: 89–94.

LAM, T. J. 1980. Thyroxine enhances larval development and survival in *Sarotherodon mossambicus* Ruppell. Aquaculture 21: 287–291.

LAM, T. J., J. V. JUARIO, AND J. BANNO. 1985. Effect of thyroxine on growth and development in post-yolk-sac larvae of milkfish, *Chanos chanos*. Aquaculture 46: 179–184.

LAM, T. J., AND R. SHARMA. 1985. Effects of salinity and thyroxine on larval survival, growth and development of the carp, *Cyprinus carpio*. Aquaculture 44: 201–212.

LEATHERLAND, J. F., P. COPELAND, J. P. SUMPER, AND R. A. SONSTEGARD. 1982. Hormonal control of gonadal maturation and development of secondary sexual characteristics in coho salmon, *Oncorhynchus kisutch*, from Lakes Ontario, Erie and Michigan. Gen. Comp. Endocrinol. 48: 196–204.

LEATHERLAND, J. F., N. E. DOWN, H. DYE, AND E. M. DONALDSON. 1989a. Changes in plasma thyroid hormone levels in pink salmon (*Oncorhynchus gorbuscha*) during their spawning migration in the Fraser River (Canada). J. Fish Biol. 35: 199–205.

LEATHERLAND, J. F., L. LIN, N. E. DOWN, AND E. M. DONALDSON. 1989b. Thyroid hormone content of eggs and early developmental stages of five *Oncorhynchus* species. Can. J. Fish. Aquat. Sci. 46: (This issue)

LEATHERLAND, J. F., AND R. A. SONSTEGARD. 1980. Seasonal changes in thyroid hyperplasia, serum thyroid hormone and lipid concentrations and pituitary gland structure in Lake Ontario coho salmon (*Oncorhynchus kisutch* Walbaum) and a comparison with coho salmon from Lakes Michigan and Erie. J. Fish Biol. 16: 539–562.

1981a. Thyroid dysfunction in Great Lakes coho salmon, *Oncorhynchus kisutch* (Walbaum): seasonal and interlake differences in serum T3 uptake and serum total and free T4 and T3 levels. J. Fish Dis. 4: 413–423.

1981b. Thyroid function, pituitary structure and serum lipids in Great Lakes coho salmon, *Oncorhynchus kisutch* Walbaum, 'jacks' compared with sexually immature spring salmon. J. Fish Biol. 18: 643–653.

1984. Pathobiological responses of feral teleosts to environmental stressors: interlake studies of the physiology of Great Lakes salmon, p. 116–149. *In* V. W. Cairns, P. V. Hodson, and J. O. Nriagu [ed.] Contaminant effects on fisheries. John Wiley & Sons Ltd., New York, NY.

1987. Comparative fecundity and egg survival in two stocks of goitred coho salmon (*Oncorhynchus kisutch* Walbaum) from Lake Erie. Can. J. Zool. 65: 2780–2785.

MACKENZIE, D. S., J. WARNER, AND P. THOMAS. 1987. Thyroid–reproductive relationships in the channel catfish, *Ictalurus punctatus*: evidence for estradiol-induced changes in plasma thyroid hormone binding, p. 205. *In* D. R. Idler, L. W. Crim, and J. M. Walsh [ed.] Reproductive physiology of fish. Memorial University Press, St. John's, Nfld.

MIWA, S., AND Y. INUI. 1987. Effects of various doses of thyroxine and triiodothyronine on metamorphosis of flounder (*Paralichthys olivaceus*). Gen. Comp. Endocrinol. 67: 356–363.

MOCCIA, R. D., J. F. LEATHERLAND, AND R. A. SONSTEGARD. 1981. Quantitative interlake comparison of thyroid pathology in Great Lakes coho salmon (*Oncorhynchus kisutch*) and chinook (*Oncorhynchus tshawytscha*) salmon. Cancer Res. 41: 2200–2210.

MORRISON, P. F., J. F. LEATHERLAND, AND R. A. SONSTEGARD. 1985. Comparative fecundity and egg survival of coho salmon (*Oncorhynchus kisutch* Walbaum) from Lakes Ontario, Erie and Michigan. Can. J. Zool. 63: 1096–1100.

NOLTIE, D., J. F. LEATHERLAND, AND M. KEENLEYSIDE. 1988. Epizootics of thyroid hyperplasia in Lake Superior and Lake Erie pink salmon (*Oncorhynchus gorbuscha* Walbaum). Can. J. Zool. 66: 0000–0000.

SOWER, S. A., AND B. SCHRECK. 1982. Steroid and thyroid hormones during sexual maturation of coho salmon (*Oncorhynchus kisutch*) in seawater or fresh water. Gen. Comp. Endocrinol. 47: 42–53.

STEEL, R. G. D., AND J. H. TORRIE. 1980. Principles and procedures of statistics. McGraw-Hill Co., New York, NY. 481 p.

SULLIVAN, C. V., R. N. IWAMOTO, AND W. W. DICKHOFF. 1987. Thyroid hormones in blood plasma of developing salmon embryos. Gen. Comp. Endocrinol. 65: 337–345.

TAGAWA, M., AND T. HIRANO. 1987. Presence of thyroxine in eggs and changes in its content during early development of chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 68: 129–135.

UEDA, H., O. HIROI, A. HARA, K. YAMAUCHI, AND Y. NAGAHAMA. 1984. Changes in serum concentrations of steroid hormones, thyroxine, and vitellogenin during spawning migration of the chum salmon, *Oncorhynchus keta*. Gen. Comp. Endocrinol. 53: 203–211.

Size Structure of a Lake Trout (*Salvelinus namaycush*) Population in an Arctic Lake: Influence of Angling and Implications for Fish Community Structure

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McDonald, M. E., and A. E. Hershey. 1989. Size structure of a lake trout (*Salvelinus namaycush*) population in an Arctic lake: influence of angling and implications for fish community structure. Can. J. Fish. Aquat. Sci. 46: 2153–2156.

The lake trout (*Salvelinus namaycush*) population in Toolik Lake in arctic Alaska was assessed by gill net during 1977, prior to exploitation. In 1986 we replicated the gill net study to determine the effects of recent angling pressure on the fish community. Relative composition of the catch varied between samples. Round whitefish (*Prosopium cylindraceum*), which are not susceptible to angling, made up 42% of the catch in 1986 and 28% in 1977. Significant reductions in the median length and weight of lake trout between the sampling periods were observed. The median size of lake trout in 1986 was below the minimum 1977 reproductive size. Differences in median length and weight of grayling (*Thymallus arcticus*) and of round whitefish during this period were not significantly different. The condition factor of lake trout was significantly higher in 1986. We suggest that large lake trout in Toolik Lake control salmonid recruitment and determine community structure. With continued fishing pressure further changes in the fish community and lake trophic dynamics may result.

La population de truites grises (*Salvelinus namaycush*) du lac Toolik dans la partie arctique de l'Alaska a été évaluée au filet maillant en 1977, avant son exploitation. En 1986, l'étude au filet maillant a été répétée afin qu'on puisse déterminer les effets de la pression exercée par la récente pêche à la ligne sur cette communauté de poisson. La composition relative de la prise a varié d'un échantillon à l'autre. Le ménomini rond (*Prosopium cylindraceum*), qui n'est pas susceptible d'être pêché à la ligne, constituait 42 % de la prise en 1986 et 28 % en 1977. On a observé des réductions importantes de la longueur et du poids médians de la truite grise entre les deux périodes d'échantillonnage. La longueur médiane observée en 1986 était inférieure à la longueur minimum à la reproduction de 1977. Il n'y avait pas de différence significative dans la longueur et le poids moyens de l'ombre arctique (*Thymallus arcticus*) et du ménomini rond durant cette période. Le facteur de condition de la truite grise était significativement supérieur en 1986. Nous avançons l'hypothèse à l'effet que la grosse truite grise du lac Toolik contrôle le recrutement des salmonidés et détermine la structure de la communauté. Sous la pression incessante de la pêche, il peut se produire de nouveaux changements dans la communauté des poissons et dans la dynamique trophique du lac.

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The regulation of lake ecosystems by predatory fishes was first suggested by Hrbáček (1962) and Brooks and Dodson (1965). The predator's regulation occurs primarily through consumption of zooplanktivorous prey fishes (Shapiro 1980; Shapiro and Wright 1984; Carpenter and Kitchell 1984). Prey fish populations in temperate systems can be highly variable (see Crowder et al. 1987; M. E. McDonald and J. H. Selgeby, unpubl. data) and this variability may also produce changes in the benthic community (McDonald et al. 1990). Experimental manipulations of food webs have been suggested as necessary to develop a fuller understanding of the role of predation (Carpenter et al. 1985).

In lakes that lack a pelagic prey fish, such as those in the Toolik Lake region of arctic Alaska, the effects of a top predator may be very different. Although juvenile salmonids play an important role in structuring these zooplankton communities (O'Brien et al. 1979a, 1979b; Schmidt and O'Brien 1982), arctic lakes appear to be dominated by adult lake trout (*Salvelinus namaycush*), with few subadult-sized fish (Johnson 1976). In the absence of a pelagic prey fish, adult lake trout in the arctic feed primarily on benthic invertebrates (O'Brien et al. 1979a; Merrick 1989) and may act as keystone predators on competing snail populations (Hershey 1989; G. Merrick and A. E. Hershey, unpubl. data). Brandt (1986) has suggested that lake trout may have acted as a keystone predator in Lake Ontario, allowing two species of sculpin to coexist. However, it is unknown whether changes in population of a lake trout in the absence of other system changes will affect the rest of the fish community.

Prior to the construction of the Alaska pipeline, access to Toolik Lake was only by charter floatplane. Fishing in Toolik

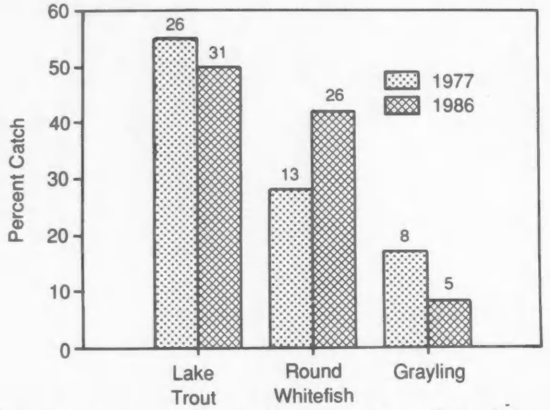

FIG. 1. Each species as a proportion of the total catch in 1977 (from O'Brien et al. 1979a) and 1986. Absolute catch of each species during each year is given above the histograms.

Lake was illegal during the construction of the pipeline. The ban on fishing was lifted in 1981 and angling pressure on Toolik became substantial. We took advantage of the recent sportfishing for lake trout in Toolik Lake to examine concomitant changes in the fish community. Preexploitation data on fork length, weight, and maturity for lake trout, grayling (*Thymallus arcticus*), and round whitefish (*Prosopium cylindraceum*) was available (see O'Brien et al. 1979a) and formed the basis of a 9-yr comparison with extant populations.

Methods

Toolik Lake is 148.6 ha and is located along the Dalton Highway in arctic Alaska (68°37′N, 149°36′W), 25 km north of the Brooks Mountains (see McDonald et al. 1982). Toolik Lake was sampled using experimental gill nets along the western shore by Alaska Fish and Game in 1977, prior to extensive fishing pressure; 47 fishes were collected (see O'Brien et al. 1979a). In 1986, we repeated Alaska Department of Fish and Game's sampling, using the same number of nets (3) of the same length and mesh size (38.1 m with five panels; a panel consisted of a single mesh of 19, 25, 38, 51, or 64 mm). These nets were set in the same location as the previous sampling and for the same period (12 h starting at 20:00) on three consecutive days. Fishes removed from the nets were identified, enumerated, weighed, and measured.

We determined median values for fork length and weight for lake trout, grayling, and round whitefish caught in 1977 (from O'Brien et al. 1979a) and in 1986. Comparisons of length and weight for each species between years were by Wilcoxon two-sample test (Steel and Torrie 1980). Condition factor (K = weight (g) $10^5 \cdot$fork length (mm)$^{-3}$; see Weatherley 1972) was calculated for each species. Differences in condition of each species between years were determined by t-test. Differences were accepted as significant if $P < 0.05$.

Results

During the 1986 gill netting, 35% more fishes were caught (62 total) than during 1977 (46 total, from O'Brien et al. 1979a). In 1986, 31 of the fishes collected were lake trout, 26 were round whitefish, and 5 were grayling (Fig. 1). This was a 5% decrease in relative catch of lake trout from 1977, a 14% increase in round whitefish, and a 9% decrease in grayling (Fig. 1).

TABLE 1. Median fork length and median weight for each fish species in 1977 and 1986. Values for 1977 and minimum reproductive size were determined from O'Brien et al. (1979a). Significant differences between years are denoted by an asterisk. LT = lake trout, RW = round whitefish, GR = grayling.

	1986		1977		1977 minimum reproductive size observed	
Species	Fork length (mm)	Weight (g)	Fork length (mm)	Weight (g)	FL	WT
LT	313*	318	388	578	365	490
RW	381	680	390	760	310	300
GR	249	185	305	380	280	305

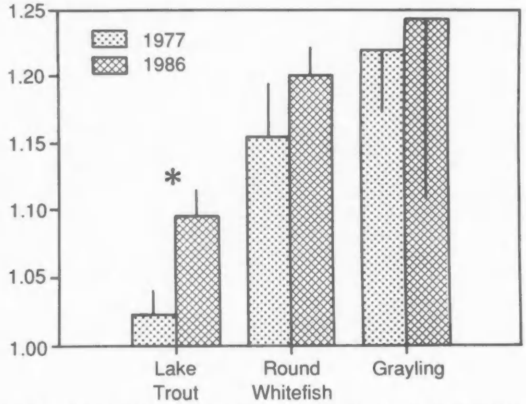

FIG. 2. Condition factor, K (± 2 SD), for each species in 1977 (determined from O'Brien et al. 1979a) and 1986. Significant differences in condition between years are denoted by an asterisk.

Lake trout in Toolik Lake showed a significant decrease in median fork length from 388 mm in 1977 to 313 mm in 1986 (Table 1). Median weight also significantly decreased from 578 to 318 g during this same period (Table 1). Both the median fork length and weight in 1986 were smaller than the minimum reproductive-sized individuals in 1977 (365 mm, 490 g; Table 1). Round whitefish were not significantly different between 1977 and 1986 in either median fork length or median weight (Table 1). Median size of round whitefish in 1986 was above the minimum reproductive size of 1977 (Table 1). Grayling had nonsignificant changes in median fork length and median weight between 1977 and 1986; median grayling size was larger than smallest reproductive size in 1977 but smaller in 1986 (Table 1).

The condition factor for lake trout was significantly higher in 1986 than in 1977 (Fig. 2). However, whitefish and grayling condition factors did not differ significantly between the two sampling periods.

Discussion

Although no species of fish caught in Toolik Lake in 1977 had been lost by 1986, the fish community appears to have shifted: round whitefish made up a much larger percentage of the catch in 1986 (42%) than they did in 1977 (28%; Fig. 1). This shift in the relative abundance of species is consistent with that expected after angling. Round whitefish are not taken by anglers and may have indirectly benefitted from the sportfishery.

The decline in lake trout median size over a 9-yr period is consistent with the expected size change of individuals in an exploited population (Rawson 1961). The median-sized lake trout in Toolik Lake in 1986 was still susceptable to angling, but was smaller than the 1977 minimum size of sexual maturity (Table 1). If angling pressure continues to deplete the mature stock, sufficient reproductive capacity to maintain the population may be lost. The loss of reproductive capacity may be extremely important because many lake trout in northern latitudes are probably intermittent spawners (Miller and Kennedy 1948; Johnson 1972, 1973), and it may require a number of years for smaller lake trout to reach reproductive size (Johnson 1976). Reduction in mature lake trout due to angling was responsible for reduced recruitment in another system (Paterson 1968). However, Healy (1978) suggested that fecundity may increase with exploitation and that this may compensate for increased fishing mortality. This assumes that there is sufficient remaining mature stock, or that a sufficient number of individuals can reach reproductive size rapidly. In unexploited arctic lakes, it is not typically possible for large numbers of lake trout to reach maturity rapidly (Johnson 1972, 1976).

Condition factors for all species appeared to improve between sampling periods, but only that of lake trout improved significantly (Fig. 2). We suggest that in 1977 the lake trout were food limited, while feeding conditions were improved in 1986. Lake trout in Toolik Lake are primarily benthivorous at a fork length of 400 mm (Merrick 1989). Preliminary bioenergetics simulation modeling suggests that lake trout larger than this may have limited capabilities to grow when feeding on benthos (M. E. McDonald and A. E. Hershey, unpubl. data). Thus, larger fish relying on benthos would have had a lower condition factor. When large lake trout are abundant, they act to reduce the density of recruiting lake trout through piscivory (Harper 1948; Miller and Kennedy 1948; Martin 1955; Johnson 1975, 1976) and/or by forcing juveniles into suboptimal habitats (Royce 1951; Johnson 1972, 1976); they may also affect round whitefish and perhaps grayling similarly. In Toolik Lake as large lake trout were removed by anglers, smaller lake trout (which are primarily zooplanktivores and/or benthivores) were able to recruit into the population. A similar response by juvenile lake trout was observed in Keyhole Lake in the Canadian Arctic after an intensive fishery (Johnson 1976).

Lake trout appear to act as ecosystem regulators. Brandt (1986) has suggested that lake trout may have acted as a keystone predator in regulating the interaction between two sculpin species. Johnson (1976) suggested that large lake trout in undisturbed systems may regulate their own recruitment. The observed change in size structure of the lake trout population compared with the increase in percent catch of round whitefish suggests that adult lake trout limit fish recruitment in Toolik Lake as well. Hershey (1989) and M. E. Merrick and A. E. Hershey (unpubl. data) have shown that lake trout may act as keystone predators on competing snail populations in arctic lakes. Lake trout in Toolik Lake appear to regulate the fish community structure as well as benthic–pelagic interactions. If sportfishing pressure continues, the regulation of the ecosystem by lake trout may break down and significant changes in the fish community and in lake trophic dynamics may result.

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References

BRANDT, S. B. 1986. Disappearance of the deepwater sculpin (*Myoxocephalus thompsoni*) from Lake Ontario: the keystone predator hypothesis. J. Great Lakes Res. 12: 18–24.

BROOKS, J. L., AND S. L. DODSON. 1965. Predation, body-size, and the composition of the plankton. Science (Wash., DC) 150: 28–35.

CARPENTER, S. R., AND J. F. KITCHELL. 1984. Plankton community structure and limnetic primary production. Am. Nat. 124: 159–172.

CARPENTER, S. R., J. F. KITCHELL, AND J. R. HODGSON. 1985. Cascading trophic interactions and lake productivity. BioScience 35: 634–639.

CROWDER, L. B., M. E. MCDONALD, AND J. A. RICE. 1987. Understanding recruitment of Lake Michigan fishes: the importance of size-based interactions between fish and zooplankton. Can. J. Fish. Aquat. Sci. 44 (Suppl. 2): 141–147.

HARPER, F. 1948. Fishes of the Nueltin Lake expedition, Keewatin, 1947. Part 2 — Historical and field notes. Proc. Acad. Nat. Sci. Phila. 50: 153–184.

HEALY, M. C. 1978. Dynamics of exploited lake trout populations and implications for management. J. Wildl. Manage. 42: 307–328.

HERSHEY, A. E. 1989. Snail populations in arctic lakes: competition mediated by predation. Oecologia.

HRBÁČEK, J. 1962. Species composition and the amount of zooplankton in relation to the fish stock. Rozpr. Cesk. Akad. Ved. Rada Mat. Prir. Ved. 72: 1–114.

JOHNSON, L. 1972. Keller Lake: characteristics of a culturally unstressed salmonid community. J. Fish. Res. Board Can. 29: 731–740.

1973. Stock and recruitment in some unexploited Canadian Arctic Lakes. Rapp. P.-V. Réun. Cons. Int. Explor. Mer 164: 219–227.

1975. Distribution of fish species in Great Bear Lake, Northwest Territories, with reference to zooplankton, benthic invertebrates, and environmental conditions. J. Fish. Res. Board Can. 32: 1989–2004.

1976. Ecology of arctic populations of lake trout, *Salvelinus namaycush*, lake whitefish, *Coregonus clupeaformis*, arctic char, *S. alpinus*, and associated species in unexploited lakes of the Canadian Northwest Territories. J. Fish. Res. Board Can. 33: 2459–2488.

MARTIN, N. V. 1955. Limnological and biological observations in the region of the Ungava or Chubb Crater, Province of Quebec. J. Fish. Res. Board Can. 12: 487–498.

MCDONALD, M. E., L. B. CROWDER, AND S. B. BRANDT. 1990. Changes in *Mysis* and *Pontoporeia* populations in southeastern Lake Michigan: a response to shifts in the fish community. Limnol. Oceanogr. (In press)

MCDONALD, M. E., B. E. CUKER, AND S. C. MOZLEY. 1982. Distribution, production and age structure of slimy sculpin in an arctic lake. Environ. Biol. Fishes 7: 171–176.

MERRICK, G. 1989. Lake trout (*Salvelinus namaycush*) and benthic community ecology in an arctic ecosystem. M.S. thesis, University of Minnesota-Duluth, Duluth, MN.

MILLER, R. B., AND W. A. KENNEDY. 1948. Observations on the lake trout of Great Bear Lake. J. Fish. Res. Board Can. 5: 176–189.

O'BRIEN, W. J., C. BUCHANON, AND J. HANEY. 1979a. Arctic zooplankton community structure: exceptions to some general rules. Arctic 32: 237–247.

O'BRIEN, W. J., D. KETTLE, AND H. RIESSEN. 1979b. Helmets and invisible armor: structures reducing predation from tactile and visual planktivores. Ecology 60: 287–294.

PATERSON, R. J. 1968. The lake trout (*Salvelinus namaycush*) of Swan Lake, Alberta. Atla. Dep. Lands For. Fish Wildl. Div. Res. Rep. 2: 1–149.

RAWSON, D. S. 1961. The lake trout of Lac la Ronge, Saskatchewan. J. Fish. Res. Board Can. 18: 423–462.

ROYCE, W. F. 1951. Breeding habits of lake trout in New York. U.S. Fish Wildl. Serv. Fish. Bull. 52: 59–76.

SCHMIDT, D., AND W. J. O'BRIEN. 1982. Planktivorous feeding ecology of arctic grayling (*Thymallus arcticus*). Can. J. Fish. Aquat. Sci. 39: 475–482.

SHAPIRO, J. 1980. The importance of trophic-level interactions to the abundance and species composition of algae in lakes, p. 105–115. *In* J. Barica and L. R. Mur [ed.] Hypertrophic ecosystems. Dr. W. Junk Publishing Co., The Hague, The Netherlands.

SHAPIRO, J., AND D. WRIGHT. 1984. Lake restoration by biomanipulation: Round Lake, Minnesota, the first two years. Freshwater Biol. 14: 371–383.

STEEL, R. G. D., AND J. H. TORRIE. 1980. Principles and procedures of statistics. McGraw-Hill Book Company, New York, NY. 633 p.

WEATHERLEY, A. H. 1972. Growth and ecology of fish populations. Academic Press, New York, NY. 293 p.

Power-Cost Efficiency of Eight Macrobenthic Sampling Schemes in Puget Sound, Washington, USA[1]

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Ferraro, S. P., F. A. Cole, W. A. DeBen, and R. C. Swartz. 1989. Power-cost efficiency of eight macrobenthic sampling schemes in Puget Sound, Washington, USA. Can. J. Fish. Aquat. Sci. 46: 2157–2165.

Power-cost efficiency (PCE$_i$ = $(n \times c)_{\min}/(n_i \times c_i)$, where i = sampling scheme, n = minimum number of replicate samples needed to detect a difference between locations with an acceptable probability of Type I (α) and Type II (β) error (e.g. $\alpha = \beta = 0.05$), c = mean "cost," in time or money, per replicate sample, and $(n \times c)_{\min}$ = minimum value of $(n \times c)$ among the i sampling schemes) is the appropriate expression for comparing the cost efficiency of alternative sampling schemes having equivalent statistical rigor when the statistical model is a t-distribution for comparisons of two means. PCEs were determined for eight macrobenthic sampling schemes (four sample unit sizes and two sieve mesh sizes) in a comparison of a reference site versus a putative polluted site in Puget Sound, Washington. Laboratory processing times were, on average, about 2.5 times greater for the ≥0.5- than the ≥1.0-mm samples. The 0.06-m^2, 0- to 8-cm-deep sample unit size and 1.0-mm sieve mesh size was the overall optimum sampling scheme in this study; it ranked first in PCE on 8 and second on 3 of 11 measures of community structure. Rank order by statistical power of the 11 measures for this scheme was Infaunal Index > $\log_{10}$ (mollusc biomass +1) > number of species > $\log_{10}$ (numerical abundance) > $\log_{10}$ (polychaete biomass + 1) > $\log_{10}$ (total biomass + 1) > $\log_{10}$ (crustacean biomass + 1) > McIntosh's Index > 1 − Simpson's Index > Shannon's Index > Dominance Index.

Pour la comparaison coût-efficacité de différents systèmes d'échantillonnage qui ont une rigueur statistique équivalente lorsque le modèle statistique est une distribution en t qui permet la comparaison de deux moyennes, l'expression appropriée est l'efficacité puissance-coût [PCE$_i$ = $(n \times c)_{\min}/(n_i \times c_i)$, où i = système d'échantillonnage, n = nombre minimum d'échantillons répétés nécessaires à la détection d'un écart entre deux emplacements avec une probabilité acceptable d'erreur du type I (α) et du type II (β) (p. ex., $\alpha = \beta = 0,05$), c = « coût » moyen, en temps ou en argent, par échantillon répété, et $(n \times c)_{\min}$ = valeur minimale de $(n \times c)$ dans les systèmes d'échantillonnage i]. Les PCE ont été déterminées au regard de huit systèmes d'échantillonnage macrobenthiques (quatre formats d'échantillons et deux maillages) lors de la comparaison d'un emplacement de référence et d'un emplacement qu'on jugeait pollué, à Puget Sound, Washington. Cependant, la durée des traitements en laboratoire était en moyenne 2,5 fois supérieure dans le cas des échantillons de ≥0,5 mm que dans celui des échantillons ≥1,0 mm. Dans cette étude, le système d'échantillonnage optimal global a été l'unité d'échantillonnage de 0,06 m^2 entre 0 et 8 cm de profondeur et le maillage de 1,0 mm; ce système s'est classé au premier rang de classement des PCE sur 8 et au deuxième rang sur 3 des 11 mesures portant sur la structure des communautés. L'ordre dans le rang par puissance statistique des 11 mesures de ce système est le suivant : indice de l'endofaune > $\log_{10}$ (biomasse des mollusques + 1) > nombre d'espèces > $\log_{10}$ (abondance numérique) > $\log_{10}$ (biomasse des polychètes + 1) > $\log_{10}$ (biomasse totale + 1) > $\log_{10}$ (biomasse des crustacés + 1) > Indice de McIntosh > 1 − Indice de Simpson > Indice de Shannon > Indice de dominance.

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The importance of determining the appropriate sampling scheme (sample unit size (area × depth) and sieve mesh size) for quantitative macrobenthic studies is often underestimated. Recommendations by Gonor and Kemp (1978), Green (1979), McIntyre et al. (1984), and others to carry out preliminary sampling to determine the best sampling methods and design are frequently unheeded. Field ecologists who rely on intuition, who simply use readily available sampling gear, or who uncritically adopt the sampling scheme of another study leave sampling efficacy and efficiency to chance.

Macrobenthic studies such as Holme (1953), Longhurst (1959), and Lie (1968) are often cited as precedents. These were faunal surveys not quantitative studies (sensu Elliott 1977). The "standard" faunal survey takes five replicate, 0.1-m^2 grabs per station. The decision for this level of sampling effort was based primarily on considerations of ship-time costs for deepwater sampling and the diminishing returns of additional samples (Longhurst 1959; Lie 1968). Many benthic ecologists working nearshore, however, have adopted this protocol for quantitative studies even though laboratory sample-processing costs are primarily responsible for the difference in cost of alternative sampling schemes (Saila et al. 1976), and data requirements of a quantitative study may not be the same as those for a typical faunal survey. Parsimony is attained in a quantitative study to detect differences in location when the minimum cost sampling scheme which is necessary and sufficient to detect a specific kind and magnitude of change is used.

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The smallest area sample unit size, for a given total area sampled, is usually the best for quantitative purposes (Cochran 1977; Elliott 1977; Green 1979; Heltshe and Ritchey 1984). There are, however, sufficient exceptions to this rule to warrant testing the appropriateness of a given sample unit size (Green 1979), and the question of what total area must be sampled to meet one's objective(s) remains. Depth of sample is important if one wishes a representative sample of the benthos. Most benthic species and individuals are in the upper 6–10 cm (Sanders 1960; Woodin 1974; Word 1976). Benthic animals tend to be concentrated nearer the surface under polluted conditions (Pearson and Rosenberg 1978).

Study objectives determine the mesh size used in macrobenthic studies, although sediment characteristics may also influence the choice when working in coarse sediments (Eleftheriou and Holme 1984). The mesh size used will influence one's interpretation of community structure (Reish 1959; McIntyre 1961; Lewis and Stoner 1981; Eleftheriou and Holme 1984; Rees 1984). Although small individuals are missed by large mesh sizes, estimates of total faunal biomass are usually accurate over the range of mesh sizes typically used in macrobenthic studies, since the mass of small organisms relative to the total tends to be small (Reish 1959; Nalepa and Robertson 1981).

The most common sieve mesh sizes used in marine macrobenthic studies are 1.0 and 0.5 mm (Rees 1984). Eleftheriou and Holme (1984) suggested, in general, using a 0.5-mm-mesh sieve for macro faunal separation, but many benthic ecologists use a 1.0-mm mesh (e.g. Dybern et al. 1976). Pollution ecologists have differing opinions on the relative merits of a 1.0- versus a 0.5-mm mesh (McIntyre et al. 1984). Hartley (1982) and McIntyre et al. (1984) questioned the cost effectiveness of using $\leq$0.5-mm-mesh screens to detect pollution-related changes in benthic communities especially when ephemeral or patchily distributed juveniles dominate the small animal fraction. In practice, "macrobenthos" is operationally defined by the sieve mesh size used. The natural distinction between "macrofauna" and "meiofauna" appears to occur somewhere between 1.0 and 0.5 mm (Reish 1959; Schwinghamer 1981).

In this paper we present a method for determining the optimum among a set of sampling schemes for detecting differences in location when the statistical model is a *t*-distribution for comparisons of two means. We define the "optimum" scheme as the least costly scheme which is capable of reliably ($\alpha = 0.05$, $1\text{-}\beta \approx 0.95$) detecting a desired difference. Following a description of the method, we work through an example showing how the method was used to determine the overall optimum sampling scheme among eight schemes to detect differences between a reference and a putative polluted site in the Everett Harbor area of Puget Sound, Washington, on 11 measures of community structure.

Description of Method

Step 1: Collect replicate samples using two or more sampling schemes at purposefully chosen paired stations, e.g. a reference and a known or presumed impacted station. The observed difference in response measures between the stations is operationally taken to be the magnitude of the difference you wish to detect. The mean difference expressed in within-population standard deviation units is the "effect size" ($\text{ES} = |\bar{X}_1 - \bar{X}_2|/\text{SD}$, where $\text{SD} = \sqrt{(s_1^2 + s_2^2)/2}$; Cohen 1977) of interest.

Step 2: Assess the "cost" (c_i), in time or money, of each sampling scheme *i* at each station.

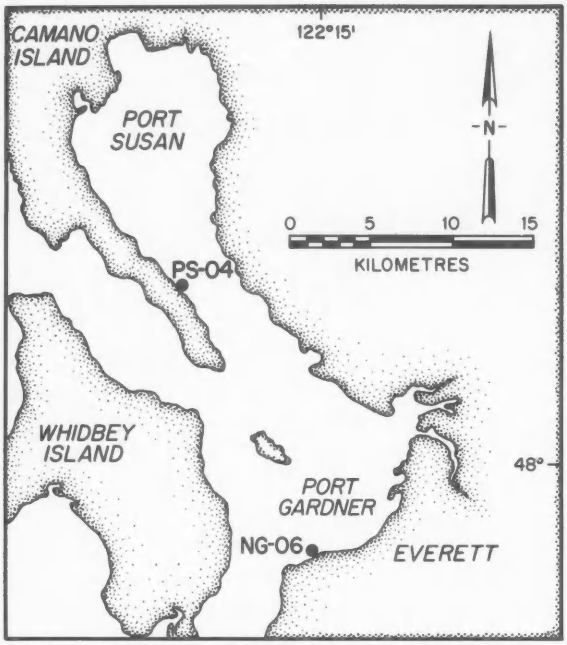

FIG. 1. Location of sampling stations in the Everett Harbor area of Puget Sound.

Step 3: Conduct statistical power analyses (Cohen 1977; Krebs 1989) to determine the minimum number of replicate samples (n_i) needed to detect the ES with an acceptable probability of Type I (α) and Type II (β) error (e.g. $\alpha = \beta = 0.05$).

Step 4: Calculate the power-cost efficiency (PCE) for each sampling scheme by

$$\text{(1)} \quad \text{PCE}_i = (n \times c)_{\min} / (n_i \times c_i)$$

where $(n \times c)_{\min}$ = minimum value of $(n \times c)$ among the *i* sampling schemes.

The PCE formula is the familiar "power efficiency" ratio of the sample sizes attained by alternative tests under similar conditions (Noether 1967; Conover 1980; Gibbons 1985) with the *n*'s multiplied by the "cost" per replicate sample. Multiplying *n* by *c* puts efficiency on a total "cost" rather than on a sample size basis. The optimum sampling scheme for a given measure has the value $(n \times c)_{\min}$. The reciprocal of PCE_i is the factor by which the optimum sampling scheme is more efficient than alternative scheme *i*. When PCE is determined for multiple measures, the overall optimum sampling scheme may be defined as that which ranks highest in PCE on most measures of interest.

Example

Materials and Methods

Macrobenthic samples were collected at a reference station in Port Susan (PS-04) and a putative polluted station in the vicinity of a fuel depot in Port Gardner (NG-06) in the Everett Harbor area of Puget Sound (Fig. 1) as a part of a study on regional pollution problems (Tetra Tech 1986a, 1986b; Tetra Tech and E. V. S. Consultants 1986). Samples were taken on 3 and 10 October 1986 from the R/V *Kittiwake* while anchored on station. Water depth (MLLW) is 8.7 and 10.2 m at stations PS-04 and NG-06, respectively, and sediment characteristics are similar at the two stations (PTI Environment Services and Tetra Tech 1988).

Ten replicate benthic samples were collected at each station with both a 0.1-m^2 van Veen grab and a 0.06-m^2 box corer. The sequence of sampling was two grabs followed by two cores to avoid variance among samplers due to vessel drift. The penetration depth was ~8 cm (range 8–9 cm) for the van Veen grab and 20–25 cm for the corer at both stations. Corer samples were partitioned into three depth strata: 0–8, 8–15, and >15 cm. All samples were sieved through 1.0- and 0.5-mm-mesh stacked screens, except the >15-cm corer samples which were only sieved through a 2.0-mm-mesh screen. Eight sampling schemes were differentiated by these collections: (A) 0.1-m^2 van Veen grab, ⩾1.0 mm, (B) 0.06-m^2 corer, 0–8 cm deep, ⩾1.0 mm, (C) 0.06-m^2 corer, 0–15 cm deep, ⩾1.0 mm, (D) 0.06-m^2 corer, 0–15 cm deep, ⩾1.0 mm + >15 cm deep, ⩾2.0 mm, (E) 0.1-m^2 van Veen grab, ⩾0.5 mm, (F) 0.06-m^2 corer, 0–8 cm deep, ⩾0.5 mm, (G) 0.06-m^2 corer, 0–15 cm deep, ⩾0.5 mm, and (H) 0.06-m^2 corer, 0–15 cm deep, ⩾0.5 mm + >15 cm deep, ⩾2.0 mm. The material retained on each sieve was placed in sample bags with 10% buffered formalin in seawater. They were transferred to glass jars and 70% isopropanol after 24–96 h. We received five van Veen and five corer samples from stations PS-04 and NG-06 for this study.

Sample volumes were measured (±5 mL) and samples were transferred to 70% ethanol and stained with rose bengal before being sorted under a dissecting microscope (6–16×). Ten percent of each sample was resorted by a different individual. If the sorting error was greater than 5% (excluding Foraminifera), the entire sample was resorted to ensure ⩾95% sorting efficiency. Sorting of Foraminifera was problematic. Live foraminiferans stain a deep rose color (Walton 1952), but foraminiferan tests are difficult to distinguish from sand grains and they break easily upon handling. Therefore, we did not ensure ⩾95% sorting efficiency for Foraminifera.

Sorted organisms were separated into major taxonomic groups (Polychaeta, Mollusca, Crustacea, "others") and placed in small containers with nitex-screen bottoms which were allowed to drain for 5 min prior to weighing. Group biomass (± 0.01 g wet wt) was determined by subtracting the container weight from the container plus sample weight. Specimens were then identified to species, where possible, and counted. Identifications of the 10 most common species of each major taxonomic group from each station and size fraction were confirmed by a second taxonomist. The time (minutes) to sort, resort, count, and identify each sample was recorded. The "cost" of each sampling scheme (A–H, above) was determined as the mean total laboratory processing time per replicate sample (c_i).

Means or jackknife statistics (Miller 1974; Zahl 1977; Heltshe and Forrester 1985) and variances of the following 11 measures of macrobenthic community structure were determined for each station and sampling scheme: (1) number of species per replicate, (2) numerical abundance of all taxa per replicate, (3) biomass of all taxa (grams wet weight) per replicate, (4) Infaunal Index (unitless, see below), (5) a Dominance Index equal to the minimum number, or fraction, of species whose combined abundance was equal to 75% of the specimens in each replicate (Swartz et al. 1985, 1986), (6) Shannon's Index, H' (Shannon and Weaver 1964) (units = decits), (7) the complement of Simpson's Index (Simpson 1949), (8) McIntosh's Index (McIntosh 1967, expression 4), (9) polychaete biomass (grams wet weight) per replicate, (10) mollusc biomass (grams wet weight) per replicate, and (11) crustacean biomass (grams wet weight) per replicate.

The Infaunal Index (also called the "Infaunal Trophic Index") was developed by Word (1978, 1980) to characterize benthic community responses to pollution in southern California. The general form of the index is

$$\text{(2)}\quad \text{Infaunal Index} = 100 - \left[C\left(\sum_{i=1,j}(i-1)N_i/\sum_{i=1,j}N_i\right)\right]$$

where N_i is the number of individuals in group i, i = 1 to j where j is the number of groups of animals, and C is a scaling factor to obtain index values from 0 to 100. Species in the j groups are chosen from the local fauna by their feeding type, habitat, and sensitivity to pollution such that high values indicate unpolluted conditions and low values indicate pollution. The Infaunal Index is geographically specific. Infaunal Index species groups were developed for Puget Sound by Thom et al. (1979), but they have since been modified; in this research we use the six-group version of the Infaunal Index for Puget Sound developed by J. Q. Word (Battelle Marine Research Laboratory, 439 W. Sequim Bay Road, Sequim, WA 98382, pers. comm.).

Mean and variance determinations for measures 1–3, 5 and 9–11 were made on the raw data and with the square root and $\log_{10}(Y)$ or $\log_{10}(Y + 1)$ transformation. Normality was tested by the Kolmogorov–Smirnov statistic and homogeneity of variance by F_{max} (Sokal and Rohlf 1981). The form of the data which best met the parametric assumption of equal variances was used in subsequent analyses.

Jackknife statistics were determined for measures 4 and 6–8 after pooling replicate samples to determine when the pooled sample contents of each sampling scheme reached an asymptotic value. The pooling procedure is a preliminary step in estimating community statistics from a sample (Pielou 1966). Our asymptote criterion was $k + 1$, where k is the minimum number of pooled replicates whose mean index value was within ±5% of the index value for all (five) pooled replicate samples; $k + 1$ was chosen to insure a stable index value and a minimum sample size of two for variance estimation. The minimum number of pooled replicates for which the jackknife estimates applied was determined as the larger of the $k + 1$ values at the two stations. These jackknife statistics are community-wide estimates and are sample size independent for $n \geqslant k + 1$. Jackknife statistics were computed using the computer program JACK (Rohlf 1982). Jackknife "pseudovalues" were tested for normality by the Kolmogorov–Smirnov statistic and jackknife variances were tested for homogeneity across stations by F_{max}.

Statistical power analyses for a two-tailed t-test were conducted using the computer program POWER (Computing Center, University of Texas, Austin, TX 78712) to determine the minimum number of replicate samples (n_i) needed to detect an ES equal to $|\bar{X}_{PS\text{-}04} - \bar{X}_{NG\text{-}06}|/\text{SD}$, where SD = $\sqrt{(s_{PS\text{-}04}^2 + s_{NG\text{-}06}^2)/2}$, for measures 1–11 and the eight sampling schemes (i = A–H) with $\alpha = 0.05$ and $1-\beta \approx 0.95$. The condition of $1-\beta$ is approximate because an integer value for n generally does not exist for $1-\beta$ exactly equal to 0.95. The n_i's were also determined for β's of ~0.20 or 10^{-7} to test the stability of PCE for different β's. A small β (10^{-7}) was chosen because n_i approaches a maximum value and the relative value of n_i's among sampling schemes tends to stabilize as β

TABLE 1. Calculation of power-cost efficiency (PCE[a]) for the number of species in this study.

Sampling scheme	n	c	$n \times c$	PCE
van Veen, ≥1.0 mm	13	36.0	468.0	0.43
Corer, 0–8 cm, ≥1.0 mm	8	25.2	201.6	1.00
Corer, 0–15 cm, ≥1.0 mm	8	40.3	322.4	0.63
Corer, 0–15 cm, ≥1.0 mm[b]	8	43.2	345.6	0.58
van Veen, ≥0.5 mm	16	101.8	1628.8	0.12
Corer, 0–8 cm, ≥0.5 mm	21	70.3	1476.3	0.14
Corer, 0–15 cm, ≥0.5 mm	21	112.6	2364.6	0.09
Corer, 0–15 cm, ≥0.5 mm[b]	22	115.5	2541.0	0.08

[a] $\mathrm{PCE}_i = (n \times c)_{\min}/(n_i \times c_i)$, where i = sampling scheme, n = minimum number of replicate samples needed to detect a difference between stations with $\alpha = 0.05$ and $\beta \approx 0.05$, c = mean laboratory processing time (hours), and $(n \times c)_{\min}$ = minimum value of $(n \times c)$ among the i sampling schemes.

[b] Plus >15 cm, ≥2.0 mm.

TABLE 2. Mean time[a] (hours) to process macrobenthic samples.

Sample unit	Sort	Resort	Identify and count	Total
≥1.0-mm size fraction				
van Veen	19.2	1.8	15.0	36.0
Corer, 0–8 cm	14.6	2.3	8.3	25.2
Corer, 0–15 cm	26.7	4.2	9.4	40.3
Corer, 0–15 cm[b]	28.8	4.5	9.9	43.2
≥0.5-mm size fraction[c]				
van Veen	56.5	4.4	40.9	101.8
Corer, 0–8 cm	40.2	4.6	25.5	70.3
Corer, 0–15 cm	76.2	7.7	28.7	112.6
Corer, 0–15 cm[b]	78.3	8.0	29.2	115.5
≥0.5-mm size fraction, excluding Foraminifera[d]				
van Veen	48.3	3.8	37.5	89.6
Corer, 0–8 cm	36.4	4.3	24.0	64.7
Corer, 0–15 cm	72.4	7.4	27.2	107.0
Corer, 0–15 cm[b]	74.5	7.7	27.7	109.9

[a] Rounded to the nearest 0.1 h.

[b] Plus >15 cm, ≥2.0 mm.

[c] Times for ≥0.5-mm size fraction = time for ≥1.0-mm size fraction + time for 1.0- to 0.5-mm size fraction.

[d] Estimated sorting times of ≥0.5-mm samples excluding foraminiferans = sorting time for ≥ 1.0-mm size fraction + sorting time for 1.0- to 0.5-mm size fraction × (total number of individuals in 1.0- to 0.5-mm size fraction − number of live foraminiferans in 1.0- to 0.5-mm size fraction)/total number of individuals in 1.0- to 0.5-mm size fraction. Foraminifera identification and counting times were subtracted from the total identification and counting times of the 1.0- to 0.5-mm samples.

approaches 0. When effect sizes were small, n_i's were not estimable for $\beta \approx 10^{-7}$, but they were for $\beta \approx 0.20$. The program POWER was also used to determine the statistical power of the 11 measures for the overall optimum sampling scheme for constant α and n.

Results of the "cost" assessments and power analyses were used to determine the PCE of the eight sampling schemes by formula (1). An example calculation of PCE for the number of species in this study is shown in Table 1. The optimum sampling scheme, i.e. the lowest "cost" scheme to achieve $1-\beta \approx 0.95$, has the minimum value of $(n \times c)$ and a PCE = 1. PCE is an expression of the relative cost efficiency of sampling schemes with equivalent statistical power; e.g. the van Veen grab, ≥1.0-mm sampling scheme was 43% as cost efficient as the corer, 0- to 8-cm-deep, ≥1.0-mm sampling scheme for reliably ($\alpha = 0.05$, $1-\beta \approx 0.95$) detecting differences in numbers of species.

Results

Sorting and resorting samples, identifying the 300 species, and counting the 38 611 organisms collected in this study took over 2000 person-hours. A few samples failed to meet our criterion of ≥95% sorting efficiency and they were resorted in their entirety. We used the species, abundance, and biomass data from the 100% resorted samples, but the time for 100% resorting was not included in the time for processing samples, since it would tend to distort the relationship of laboratory processing times among sampling schemes. Small mollusc shells had dissolved in the 0.5-mm size fraction samples even though standard fixing and preservation procedures were used (Tetra Tech 1986a, 1986b). We were, therefore, only able to identify many of the small molluscs in the 1.0- to 0.5-mm size fraction samples to Class.

Our "cost" assessments (Table 2) demonstrate the influence of the area and depth of sample, and sieve mesh size on laboratory processing times. The difference in laboratory processing time between the 0.1-m^2 van Veen grab and the 0.06-m^2 corer, 0- to 8-cm-deep samples at each station was approximately in proportion to their areas, as one would expect, for samples to the same depth. It took, on average, about 2.5 times as long to process ≥0.5-mm samples as ≥1.0-mm samples, and c_i was not significantly related to sample volume (Fig. 2). To our knowledge, these are the first published data on the impact of both sample unit size and sieve mesh size on laboratory processing times for marine macrobenthic samples. Sample heterogeneity, including differences in faunal abundance and composition and in the type and amount of extraneous material in the samples, and differences in the sorting and taxonomic skills of our laboratory staff, imparted sufficient variability on c_i to mask any relationship between c_i and sample volume. Laboratory processing times will vary, depending on personnel, but the relative mean processing times for the eight schemes should be fairly constant. A convenient property of formula (1), and one which imparts greater generality to our PCE results, is that it is the ratio of c_i's, not their actual values, which determines PCEs.

Summary statistics used in the power determinations of n_i for our 11 measures of community structure are presented in Table 3. Ninety-three percent of the means and jackknife statistics passed the normality test and 83% of the variances passed the homogeneity test. Violations of the parametric assumptions were rare among the sampling schemes with high PCEs. Where they did occur they probably had only a minor effect on the power estimates of n_i, since t-tests are robust when sample sizes are equal (Glass et al. 1972; Underwood 1981). The $k + 1$ values, which served as a lower limit for n_i's, were 2 for the Infaunal, 1 − Simpson's, and McIntosh's Index and either 3 or 4 for H'.

The 0.06-m^2 corer, 0- to 8-cm-deep, ≥1.0-mm sampling scheme was the best overall among the eight schemes tested for detecting differences in community structure; it ranked first in PCE on 8 and second on 3 of the 11 measures for the complete data set and first on 5 and second on 4 of the measures when

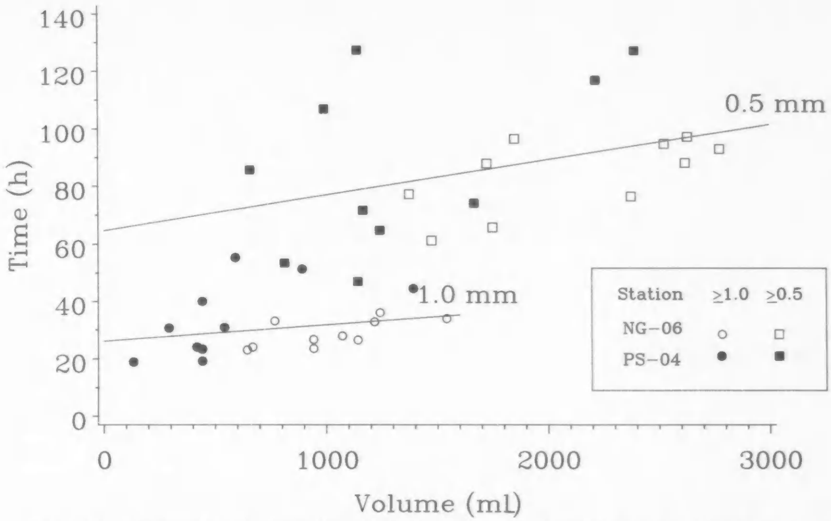

FIG. 2. Total laboratory processing times (Y) plotted as a function of the sample volume (X). Least-squares linear regression equations are $Y = 26.04 + 0.006X$ and $Y = 64.68 + 0.012X$ for the ≥1.0- and ≥0.5-mm size fraction samples, respectively. Slopes of both regressions are not significantly different from 0 ($p > 0.05$).

foraminiferans were excluded (Table 4). The 0.1-m^2 van Veen, ≥1.0-mm scheme was a distant second. The only other scheme to rank first in PCE on a measure (H') for the complete data set was the corer, 0- to 8-cm, ≥0.5-mm scheme. Rarely did the rank order of PCE's change from those in Table 4 for n_i's calculated for β's of approximately 0.20 or 10^{-7}, and never was the change more than 1 rank.

PCE was always higher in the 0- to 8-cm than in the 0- to ≥15-cm-deep corer samples for a given mesh size fraction (Table 4). Even if "cost" is ignored, the statistical power of the 0- to 8-cm-deep corer samples was usually as good or better than the deeper corer samples (e.g. see n's in Table 1 (higher n's to achieve the same $1-\beta$ indicate lower $1-\beta$ for the same n)). The information contained in the deeper samples, therefore, usually did not improve our ability to discriminate differences in community structure.

The PCE results were robust for all but four of the measures when foraminiferans were excluded from the data set. The corer, 0- to 8-cm, ≥0.5-mm scheme ranked first in PCE on H', 1 − Simpson's, McIntosh's, and the Dominance Index when foraminiferans were excluded (Table 4B). The principal effect of excluding foraminiferans was to increase the mean difference between stations (i.e. the numerator of ES) in the four diversity indices in the ≥0.5-mm corer samples, thereby decreasing n_i.

Rank order by statistical power of the 11 measures of community structure for the corer, 0- to 8-cm-deep, ≥1.0-mm sampling scheme was Infaunal Index (4, 4)[2] > $\log_{10}$ (mollusc biomass + 1) (5, 7) > number of species (5, 8) > $\log_{10}$ (numerical abundance) (5, 8) > $\log_{10}$ (polychaete biomass + 1) (5, 8) > $\log_{10}$ (total biomass + 1) (6, 9) > $\log_{10}$ (crustacean biomass + 1) (7, 10) > McIntosh's Index (7, 11) > 1 − Simpson's Index (11, 16) > H' (19, 31) > Dominance Index (40, 63).

Discussion

These study results should not be extrapolated to other locations or uncritically applied to meet other objectives at the study site. Our purpose in conducting the research in the example was to make an informed decision as to the best among eight sampling schemes for detecting pollution-related changes in macrobenthic community structure in the Everett Harbor area of Puget Sound. No single sampling scheme will be optimal in all locations, habitats, and for all study objectives. A universally applicable quantitative macrobenthic sampling scheme is an unattainable goal (Gray 1981).

Many measures have been used to quantify community structure (e.g. Washington 1984). Number of species, numerical abundance, and biomass are simple, informative, and easily interpretable measures commonly used in pollution studies (Pearson and Rosenberg 1978; Green 1979; Gray 1981). The statistical power of the 11 measures in our example reflects their sensitivity to detect the difference between stations PS-04 and NG-06. We regard this as a moderate difference. The relative sensitivities of the measures will vary for different station contrasts due to the nonlinear pollution response curves of some measures (Pearson and Rosenberg 1978), but, in general, n_i's will decrease on most measures as deviations from reference conditions (i.e. pollution effects) increase. A strategy of five replicate 0.06-m^2 corer, 0- to 8-cm-deep, ≥1.0-mm samples per station, which was sufficient for reliable ($1-\beta \approx 0.80$) detection of differences on five measures in this study, is, therefore, also likely to be sufficient for detecting moderate or greater deviations from reference conditions in Everett Harbor on at least some of our 11 measures.

The two most sensitive measures in our example (Infaunal Index and $\log_{10}$ (mollusc biomass + 1)) were also the two most sensitive measures for the same sampling scheme in a com-

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[2] Numbers in parentheses are the minimum number of replicate samples needed to detect a difference between the means or jackknife statistics of the community structure measures at stations PS-04 and NG-06 with $\alpha = 0.05$ and $1-\beta$'s of approximately 0.80 and 0.95, respectively.

TABLE 3. Summary statistics (mean or jacknife (JK) and mean s^2) for 11 measures of macrobenthic community structure at stations PS-04 and NG-06 by sample type and animal size fraction. (A) van Veen, ⩾1.0 mm; (B) corer, 0–8 cm, ⩾1.0 mm; (C) corer, 0–15 cm, ⩾1.0mm; (D) corer, 0–15 cm, ⩾1.0 mm + >15 cm, ⩾2.0 mm; (E) van Veen, ⩾0.5 mm; (F) corer, 0–8 cm, ⩾0.5 mm; (G) corer, 0–15 cm, ⩾0.5 mm; (H) corer, 0–15 cm, ⩾0.5 mm + >15 cm, ⩾2.0 mm; (I) van Veen, ⩾0.5 mm, excluding Foraminifera; (J) corer, 0–8 cm, ⩾0.5 mm, excluding Foraminifera; (K) corer, 0–15 cm, ⩾0.5 mm, excluding Foraminifera; (L) corer, 0–15 cm, ⩾0.5 mm + >15 cm, ⩾2.0 m, excluding Foraminifera.

	Number of species			$\log_{10}$ (abundance)			$\log_{10}$ (total biomass + 1)			Infaunal Index			H'			1 − Simpson's Index			McIntosh's Index			Dominance Index			$\log_{10}$ (polychaete biomass + 1)			$\log_{10}$ (mollusc biomass + 1)			$\log_{10}$ (crustacean biomass + 1)		
	PS-04 mean	NG-06 mean	Mean s^2	PS-04 mean	NG-06 mean	Mean s^2	PS-04 mean	NG-06 mean	Mean s^2	PS-04 JK	NG-06 JK	Mean s^2	PS-04 JK	NG-06 JK	Mean s^2	PS-04 JK	NG-06 JK	Mean s^2	PS-04 JK	NG-06 JK	Mean s^2	PS-04 mean	NG-06 mean	Mean s^2	PS-04 mean	NG-06 mean	Mean s^2	PS-04 mean	NG-06 mean	Mean s^2	PS-04 mean	NG-06 mean	Mean s^2
(A)	64.8	89.0	282.60	[illegible]	3.090	0.0127	0.772	0.908	0.0137	80.79	75.00	1.649	1.471	1.429	0.0168	0.935	0.908	0.0006[b]	0.755	0.698	0.0017[b]	13.05	12.22	22.676	0.537	0.611	0.0284[b]	0.364	0.753	0.0203	0.097	0.214	0.0015
(B)	49.4	67.0[a]	75.15	2.454	2.611	0.0107	0.608	0.777	0.0090	83.11[a]	75.92	6.253	1.495	1.405	0.0098	0.936	0.911	0.0004	0.763	0.707	0.0012	13.07	11.74	4.569	0.380	0.556	0.0082	0.191	0.447	0.0140	0.081	0.115	0.0004
(C)	53.8	70.6	66.00	2.492	2.678	0.0081	0.840	0.842	0.0206	83.20	75.59	7.613	1.505	1.433	0.0091	0.937	0.916	0.0004	0.757	0.714	0.0016	13.65	13.00	5.129	0.641	0.637	0.0306	0.209	0.450	0.0133[b]	0.117	0.146	0.0006
(D)	54.4	71.0	65.15	2.493	2.681	0.0078	0.954	0.851	0.0185	83.15	75.45	7.590	1.506	1.437	0.0089	0.936	0.918	0.0004	0.760	0.719	0.0015	13.73	13.13	4.945	0.642	0.651	0.0301	0.209	0.450	0.0133[b]	0.230[a]	0.146	0.0270[b]
(E)	102.6[a]	126.4	323.80	3.294	3.502	0.0061	0.814	1.027	0.0113	78.65	75.05	0.897	1.432	1.544[a]	0.0096	0.922	0.945	0.0004	0.723	0.763	0.0015	11.87	14.19[a]	18.832[b]	0.573	0.637	0.0286[b]	0.370	0.763	0.0189	0.128	0.262	0.0015
(F)	90.4	100.6	87.30	3.033	3.096	0.0123[b]	0.648[a]	0.802	0.0088	79.99[a]	75.43	1.691	1.557	1.482	0.0016	0.944	0.921	0.0002	0.766	0.726	0.0006	15.25	14.49	1.384	0.420	0.578	0.0078	0.195	0.454	0.0137	0.104	0.147	0.0008
(G)	95.8	105.0[a]	68.85[b]	3.057	3.116	0.0112	0.870	0.865	0.0196	79.62	75.28	1.844	1.571	1.497	0.0015	0.946	0.925	0.0002	0.769	0.733	0.0006	15.87	15.10	1.838	0.669	0.657	0.0275	0.215	0.457	0.0131[b]	0.144	0.177	0.0006
(H)	96.2	105.2[a]	69.95[b]	3.057	3.117	0.0111	0.977	0.874	0.0181	79.59	75.20	1.890	1.573	1.500	0.0015	0.946	0.925	0.0002	0.769	0.731	0.0006	15.91	15.22	2.051	0.670	0.671	0.0270	0.215	0.457	0.0131[b]	0.249[a]	0.177	0.0267[b]
(I)	99.0[a]	119.0	328.75	3.187	3.453	0.0068	0.803	1.026	0.0114				1.506	1.512[a]	0.0108[b]	0.940	0.935	0.0003	0.759	0.753	0.0010	14.58	13.39[a]	20.685									
(J)	87.0	97.0	89.25	2.957	3.070	0.0126[b]	0.641[a]	0.801	0.0089				1.609	1.449	0.0014	0.955	0.914	0.0001	0.789	0.710	0.0003	17.12	13.76	2.823									
(K)	92.4	101.2[a]	76.25[b]	2.979	3.084	0.0113[b]	0.865	0.854	0.0196				1.629	1.472	0.0014	0.957	0.915	0.0001	0.797	0.715	0.0003	18.07	14.58	3.240									
(L)	92.8	101.4[a]	78.00[b]	2.979	3.085	0.0111[b]	0.973	0.873	0.0184				1.632	1.474	0.0014	0.957	0.920	0.0001	0.795	0.718	0.0003	18.12	14.70	3.416									

[a]Probability of normal distribution <0.05.
[b]Probability of homogeneous variances <0.05.

parison of a reference site and a polluted site in the Southern California Bight (Ferraro et al., unpubl. data). Rank order by sensitivity of the nine other measures common to both studies, however, did not match so well. We would expect greater commonality in community response, and, therefore, a better matching of the sensitivity of the response measures, in similar habitats under similar pollution conditions. If the sensitivity of a set of measures is characteristic of a particular magnitude and type of perturbation in a geographic region and habitat (e.g. mollusc biomass > polychaete biomass > crustacean biomass indicative of moderate, type A pollution), it may be possible to predict causes from the sensitivity of the effects. Even if the order of sensitivity is not always predictive, it is useful to know which measures are more likely to change in a given situation.

The Infaunal Index has been used primarily in the Southern California Bight (Mearns and Word 1982; Swartz et al. 1985, 1986), where it has been shown to be a sensitive and robust measure of biological change (Word et al. 1980). Its sensitivity is not surprising, since it was designed, as are most biotic indices, to reflect responses to pollution (Washington 1984). The large number of taxa in the Infaunal Index species groups for southern California (53) and Puget Sound (163) makes it robust. Our results, along with those of Thom et al. (1979), indicate that the Infaunal Index is also a sensitive and robust measure of macrobenthic community differences at some locations in Puget Sound.

There are few published studies on the statistical power and cost of macrobenthic sampling schemes. Tetra Tech (1985) used a 0.06-m^2 van Veen grab in a study of the macrobenthos in Commencement Bay and subsequently recommended it as a cost-savings alternative to a 0.1-m^2 van Veen grab, although no comparison was made of either the cost or the statistical power of samples from the two grabs. Studies on the density and biomass of macrobenthic populations (Livingston et al. 1976; Downing 1979; Pringle 1984; Downing and Anderson 1985; Morin 1985; Vézina 1988) have shown that statistical precision is often improved, for a given total area (or volume) sampled, by more replication with smaller sample units. These results are a consequence of the statistical properties of the distributions of the populations studied (Elliott 1977; Green 1979). The distributions of several of our community structure measures, however, are not well defined (Bowman et al. 1971; Smith 1982). Furthermore, at some point, the "small sample" rule breaks down because as sample unit size decreases, sampler edge effects become an increasingly important source of sampling error (Elliott 1977). Sample units of area <0.1 m^2 have been found to be more power-cost efficient than 0.1-m^2 area samples even without the constraint of equal total area sampled. Michael et al. (1981), for example, found that six replicate 0.1-m^2 van Veen and six replicate 0.04-m^2 van Veen grab samples had essentially the same statistical power to detect changes in the number of species, numerical abundance, and H' at a station on Georges Bank.

Our statistical model was a t-distribution for a priori comparisons between means. This point estimation model is appropriate for determining the optimum sampling scheme for detecting differences in location in time or space (main sequences 2 and 4 in Green 1979). Cuff and Coleman (1979), Mar et al. (1985), and Millard and Lettenmaier (1986) did not address the sample unit size issue, and Cuff and Coleman's results are for a universe estimation model (Green 1980). Saila et al. (1976) used a point estimation approach on population-level responses

TABLE 4. (A) Power-cost efficiency (rank order 1–3) of eight macrobenthic sampling schemes for 11 measures of community structure: (1) number of species; (2) $\log_{10}$ (numerical abundance); (3) $\log_{10}$ (total biomass + 1); (4) Infaunal Index; (5) H'; (6) 1 − Simpson's Index; (7) McIntosh's Index; (8) a Dominance Index; (9) $\log_{10}$ (polychaete biomass + 1); (10) $\log_{10}$ (mollusc biomass + 1); (11) $\log_{10}$ (crustacean biomass + 1). (B) Same as Table 4A for measures 5–8 but also for ≥ 0.5-mm samples excluding Foraminifera.

(A) Sampling scheme	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)
van Veen, ≥1.0 mm	0.43	1.00 (1)	0.78 (2)	0.70 (2)	0.07	0.53 (3)	0.55 (2)	<0.01	0.04 (3)	0.98 (2)	1.00 (1)
Corer, 0–8 cm, ≥1.0 mm	1.00 (1)	0.71 (2)	1.00 (1)	1.00 (1)	0.72 (2)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)	0.57 (2)
Corer, 0–15 cm, ≥1.0 mm	0.63 (2)	0.45	<0.01	0.50 (3)	0.32	0.44	0.31	<0.01	<0.01	0.55 (3)	0.20
Corer, 0–15 cm, ≥1.0 mm[a]	0.58 (3)	0.48 (3)	0.12	0.47	0.28	0.30	0.28	<0.01	<0.01	0.51	0.03
van Veen, ≥0.5 mm	0.12	0.29	0.28	0.25	0.29	0.21	0.11	0.19	0.01	0.43	0.29 (3)
Corer, 0–8 cm, ≥0.5 mm	0.14	0.03	0.32 (3)	0.36	1.00 (1)	0.57 (2)	0.39 (3)	0.39 (2)	0.32 (2)	0.36	0.17
Corer, 0–15 cm, ≥0.5 mm	0.09	0.01	<0.01	0.22	0.63 (3)	0.30	0.21	0.19 (3)	<0.01	0.20	0.09
Corer, 0–15 cm, ≥0.5 mm[a]	0.08	0.01	0.05	0.22	0.61	0.29	0.22	0.13	<0.01	0.19	0.01

(B) Sampling scheme	(5)	(6)	(7)	(8)
van Veen, ≥1.0 mm	0.03	0.34	0.51	<0.01
Corer, 0–8 cm, ≥1.0 mm	0.33	0.64 (2)	0.93 (2)	0.33
Corer, 0–15 cm, ≥1.0 mm	0.15	0.28	0.29	<0.01
Corer, 0–15 cm, ≥1.0 mm[a]	0.13	0.19	0.26	<0.01
van Veen, ≥0.5 mm	0.13	0.13	0.11	0.06
Corer, 0–8 cm, ≥0.5 mm	0.46	0.37	0.37	0.13
Corer, 0–15 cm, ≥0.5 mm	0.29	0.19	0.19	0.06
Corer, 0–15 cm, ≥0.5 mm[a]	0.28	0.19	0.20	0.04
van Veen, ≥0.5 mm[b]	<0.01	0.01	<0.01	<0.01
Corer, 0–8 cm, ≥ 0.5 mm[b]	1.00 (1)	1.00 (1)	1.00 (1)	1.00 (1)
Corer, 0–15 cm, ≥0.5 mm[b]	0.60 (2)	0.60 (3)	0.60 (3)	0.60 (2)
Corer, 0–15 cm, ≥0.5 mm[ab]	0.59 (3)	0.59	0.59	0.59 (3)

[a] Plus >15 cm, ≥2.0 mm.

[b] Excluding Foraminifera. Results are presented both including and excluding Foraminifera for those measures affected by Foraminifera because they are often either excluded or are not identified to species in most benthic studies and because of the problems encountered sorting Foraminifera.

for a single sample unit size and a single mesh size, but their calculations of n_i were flawed (Millard 1987).

Our results show that macrobenthic sampling schemes based on "standard" faunal survey designs or "precedents" may not be optimal for quantitative studies. While some practical considerations may legitimately limit the feasible set of macrobenthic sampling schemes, convenience should not be the principal criterion for choosing a sampler or sieve mesh size. When the feasible set is >1, there is a potential for optimizing power-cost efficiency.

We recommend conducting a preliminary study to test alternative sampling schemes varying in ways likely to influence costs and statistical power on responses of interest whenever information is not already available to make an assessment of the relative merits of alternative schemes. If an investigator finds that the projected cost of a study using the best sampling scheme is excessive, he/she may wish to reevaluate and adjust either the data quality objectives (i.e. increase the ES or acceptable error (α, β)) or other aspects of the study design (e.g. decrease the number of stations or sampling events), or should drop the study (Eberhardt 1978). Ineffective, low-power schemes should always be discarded, since all the cost and effort is wasted. Using the sampling scheme which is both effective and least costly is most important in large studies where the consequences of choosing an inefficient scheme are greatest. In benthic pollution studies, sample unit size and sieve mesh size are important, since the distribution and size of benthic animals tend to change in response to pollution (Pearson and Rosenberg 1978). Obviously, the sampling scheme which most efficiently collects those components of the community most affected by pollution will be optimal for detecting pollution-related changes.

In summary, optimization procedures can be used on data from a preliminary study to find a statistically rigorous, cost-effective sampling scheme. Taking these steps enhances the scientific validity and can lead to substantially reduced costs of quantitative macrobenthic studies.

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References

BOWMAN, K. O., K. HUTCHESON, E. P. ODUM, AND L. R. SHENTON. 1971. Comments on the distribution of indices of diversity, p. 315–366. *In* G. P. Patil, E. C. Pielou, and W. E. Waters [ed.] Statistical ecology. Vol. 3. Many species populations, ecosystems, and systems analysis. Pennsylvania State University Press, University Park, PA.

COCHRAN, W. G. 1977. Sampling techniques. 3rd ed. John Wiley & Sons, New York, NY. 428 p.

COHEN, J. 1977. Statistical power analysis for the behavioral sciences. Revised ed. Academic Press, Orlando, FL. 474 p.

CONOVER, W. J. 1980. Practical nonparametric statistics. 2nd ed. John Wiley & Sons, New York, NY. 493 p.

Cuff, W., and N. Coleman. 1979. Optimal survey design: lessons from a stratified random sample of macrobenthos. J. Fish. Res. Board Can. 36: 351–361.

Downing, J. A. 1979. Aggregation, transformation, and the design of benthos sampling programs. J. Fish. Res. Board Can. 36: 1454–1463.

Downing, J. A., and M. R. Anderson. 1985. Estimating the standing biomass of aquatic macrophytes. Can. J. Fish. Aquat. Sci. 42: 1860–1869.

Dybern, B. I., H. Ackefors, and R. Elmgren. 1976. Recommendations on methods for marine biological studies in the Baltic Sea. Mar. Biol. 1: 98 p.

Eberhardt, L. L. 1978. Appraising variability in population studies. J. Wildl. Manage. 42: 207–238.

Eleftheriou, A., and N. A. Holme. 1984. Macrofauna techniques, p. 140–216. *In* N. A. Holme and A. D. McIntyre [ed.] Methods for the study of marine benthos. IBP Handbook No. 16. 2nd ed. Blackwell Scientific Publications, Oxford.

Elliott, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. 2nd ed. Sci. Publ. No. 25. Freshwater Biological Association, Ferry House, U.K. 159 p.

Gibbons, J. D. 1985. Nonparametric statistical inference. 2nd ed. Marcel Dekker, New York, NY. 408 p.

Glass, G. V., P. D. Peckham, and J. R. Sanders. 1972. Consequences of failure to meet assumptions underlying the fixed effects analysis of variance and covariance. Rev. Educ. Res. 42: 237–288.

Gonor, J. J., and P. F. Kemp. 1978. Procedures for quantitative ecological assessments in intertidal environments. EPA-600/3-78-087. U.S. Environmental Protection Agency, Corvallis Environmental Research Laboratory, Corvallis, OR. 104 p.

Gray, J. S. 1981. The ecology of marine sediments. An introduction to the structure and function of benthic communities. Cambridge University Press, Cambridge, U.K. 185 p.

Green, R. H. 1979. Sampling design and statistical methods for environmental biologists. John Wiley & Sons, New York, NY. 257 p.

1980. Comment on optimal survey design. Can. J. Fish. Aquat. Sci. 37: 296.

Hartley, J. P. 1982. Methods for monitoring offshore macrobenthos. Mar. Pollut. Bull. 13: 150–154.

Heltshe, J. F., and N. E. Forrester. 1985. Statistical evaluation of the jackknife estimate of diversity when using quadrat samples. Ecology 66: 107–111.

Heltshe, J. F., and T. A. Ritchey. 1984. Spatial pattern detection using quadrat samples. Biometrics 40: 877–885.

Holme, N. A. 1953. The biomass of the bottom fauna in the English Channel off Plymouth. J. Mar. Biol. Assoc. U.K. 32: 1–49.

Krebs, C. J. 1989. Ecological methodology. Harper and Row, New York, NY. 654 p.

Lewis, F. G. III, and A. W. Stoner. 1981. An examination of methods for sampling macrobenthos in seagrass meadows. Bull. Mar. Sci. 31: 116–124.

Lie, U. 1968. A quantitative study of benthic infauna in Puget Sound, Washington, USA, in 1963–1964. Fiskeridir. Skr. Ser. Havunders. 14: 229–556.

Livingston, R. J., R. S. Lloyd, and M. S. Zimmerman. 1976. Determination of sampling strategy for benthic macrophytes in polluted and unpolluted coastal areas. Bull. Mar. Sci. 26: 569–575.

Longhurst, A. R. 1959. The sampling problem in benthic ecology. Proc. N.Z. Ecol. Soc. 6: 8–12.

Mar, B. W., D. P. Lettenmaier, R. R. Horner, J. S. Richey, R. N. Palmer, S. P. Millard, M. C. MacKenzie, S. Vega-Gonzalez, and J. R. Lund. 1985. Sampling design for aquatic ecological monitoring. Vol. 1: Summary Report. Electric Power Research Institute Project 1729-1, Department of Civil Engineering, University of Washington, Seattle, WA. (Available from Research Reports Center, Box 50490, Palo Alto, CA 94303)

McIntosh, R. P. 1967. An index of diversity and the relation of certain concepts to diversity. Ecology 48: 392–404.

McIntyre, A. D. 1961. Quantitative differences in the fauna of boreal mud associations. J. Mar. Biol. Assoc. U.K. 41: 599–616.

McIntyre, A. D., J. M. Elliott, and D. V. Ellis. 1984. Introduction: design of sampling programmes, p. 1–26. *In* N. A. Holme and A. D. McIntyre [ed.] Methods for the study of marine benthos. IBP Handbook No. 16. 2nd ed. Blackwell Scientific Publications, Oxford.

Mearns, A. J., and J. Q. Word. 1982. Forecasting effects of sewage solids on marine benthic communities, p. 495–512. *In* G. F. Mayer [ed.] Ecological stress and the New York Bight: science and management. Estuarine Research Federation, Columbia, SC.

Michael, A. D., R. A. McGrath, and C. D. Long. 1981. Benthic grab comparability study. Final Report. Contract No. AA851-CT1-69. 33 p. + Appendix. (Available from Atlantic OCS Regional Office, Office of Minerals Management Service, 1951 Kidwell Drive, Vienna, VA 22180)

Millard, S. P. 1987. Environmental monitoring, statistics and the law: room for improvement. Am. Stat. 41: 249–253.

Millard, S. P., and D. P. Lettenmaier. 1986. Optimal design of biological sampling programs using the analysis of variance. Estuarine Coastal Shelf Sci. 22: 637–656.

Miller, R. G. 1974. The jackknife — a review. Biometrika 61: 1–15.

Morin, A. 1985. Variability of density estimates and the optimization of sampling programs for stream benthos. Can. J. Fish. Aquat. Sci. 42: 1530–1534.

Nalepa, T. F., and A. Robertson. 1981. Screen mesh size affects estimates of macro- and meio-benthos abundance and biomass in the Great Lakes. Can. J. Fish. Aquat. Sci. 38: 1027–1034.

Noether, G. E. 1967. Elements of nonparametric statistics. John Wiley & Sons, New York, NY. 104 p.

Pearson, T. H., and R. Rosenberg. 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr. Mar. Biol. Annu. Rev. 16: 229–311.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. J. Theor. Biol. 13: 131–144.

Pringle, J. D. 1984. Efficiency estimates for various quadrat sizes used in benthic sampling. Can. J. Fish. Aquat. Sci. 41: 1485–1489.

PTI Environmental Services and Tetra Tech. 1988. Everett Harbor Action Program: analysis of toxic problem areas. TC-3338-26. Appendix A, 2 p., and Appendix E, 2 p. (Available from PTI Environmental Services, 3625 132nd Ave. SE, Suite 301, Bellevue, WA 98006)

Rees, H. L. 1984. A note on mesh selection and sampling efficiency in benthic studies. Mar. Pollut. Bull. 15: 225–229.

Reish, D. J. 1959. A discussion of the importance of the screen size in washing quantitative marine bottom samples. Ecology 40: 307–309.

Rohlf, F. J. 1982. BIOM a package of statistical programs. Department of Ecology and Evolution, SUNY at Stony Brook, New York, NY 11794.

Saila, S. B., R. A. Pikanowski, and D. S. Vaughan. 1976. Optimum allocation strategies for sampling benthos in the New York Bight. Estuarine Coastal Mar. Sci. 4: 119–128.

Sanders, H. L. 1960. Benthic studies in Buzzards Bay. III. The structure of the soft-bottom community.-Limnol. Oceanogr. 5: 138–153.

Schwinghamer, P. 1981. Characteristic size distributions of integral benthic communities. Can. J. Fish. Aquat. Sci. 38: 1255–1263.

Shannon, C. E., and W. Weaver. 1964. The mathematical theory of communication. University of Illinois Press, Urbana, IL. 125 p.

Simpson, E. H. 1949. Measurement of diversity. Nature (Lond.) 163: 688.

Smith, E. P. 1982. The statistical properties of biological indices of water quality. Ph.D. thesis, University of Washington, Seattle, WA. 176 p.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. The principles and practice of statistics in biological research. 2nd ed. W. H. Freeman and Company, San Francisco, CA. 859 p.

Swartz, R. C., F. A. Cole, D. W. Schults, and W. A. DeBen. 1986. Ecological changes in the Southern California Bight near a large sewage outfall: benthic conditions in 1980 and 1983. Mar. Ecol. Prog. Ser. 31: 1–13.

Swartz, R. C., D. W. Schults, G. R. Ditsworth, W. A. DeBen, and F. A. Cole. 1985. Sediment toxicity, contamination, and macrobenthic communities near a large sewage outfall, p. 152–175. *In* T. P. Boyle [ed.] Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems. ASTM STP 865, American Society for Testing and Materials, Philadelphia, PA.

Tetra Tech. 1985. Summary report for the Commencement Bay nearshore/tideflats remedial investigation. EPA-910/9-85-134a. 92 p. (Available from Tetra Tech, Inc., 11820 Northup Way, Suite 100, Bellevue, WA 98005)

1986a. Sampling and analysis design for development of Everett Harbor Action Program. Draft Rep. TC 3991-03. 44 p. + 12 maps (Available from Tetra Tech, Inc., 11820 Northup Way, Suite 100, Bellevue, WA 98005)

1986b. Quality assurance project plan for field investigations to support development of the Everett Harbor Action Plan. 60 p. (Available from Tetra Tech, Inc., 11820 Northup Way, Suite 100, Bellevue, WA 98005)

Tetra Tech and E. V. S. Consultants. 1986. Cruise summary report. Benthos, bioassay and sediment chemistry survey September 30 to October 15, 1986. TC3338-01. (Available from Tetra Tech, Inc., 11820 Northup Way, Suite 100, Bellevue, WA 98005)

Thom, R. M., K. K. Chew, and J. Q. Word. 1979. Abundance, biomass, and trophic structure of the subtidal infaunal communities of the eastern side of Central Puget Sound. 43 p. (Available from the Municipality of Metropolitan Seattle, 821 Second Ave., Seattle, WA 98104)

UNDERWOOD, A. J. 1981. Techniques of analysis of variance in experimental marine biology and ecology. Oceanogr. Mar. Biol. Annu. Rev. 19: 513–605.

VÉZINA, A. F. 1988. Sampling variance and the design of quantitative surveys of the marine benthos. Mar. Biol. (Berl.) 97: 151–155.

WALTON, W. R. 1952. Techniques for recognition of living Foraminifera. Contrib. Cushman Found. Foraminiferal Res. 3: 56–60.

WASHINGTON, H. G. 1984. Diversity, biotic and similarity indices. A review with special relevance to aquatic ecosystems. Water Res. 18: 653–694.

WOODIN, S. A. 1974. Polychaete abundance patterns in a marine soft-sediment environment: the importance of biological interactions. Ecol. Monogr. 44: 171–187.

WORD, J. Q. 1976. Biological comparison of grab sampling devices. South. Calif. Coastal Water Res. Proj. Annu. Rep. 1976: 189–194.

1978. The Infaunal Trophic Index. South. Calif. Coastal Water Res. Proj. Annu. Rep. 1978: 19–39.

1980. Classification of benthic invertebrates into infaunal trophic index feeding groups. South. Calif. Coastal Water Res. Proj. Biennial Rep. 1979–1980: 103–121.

WORD, J. Q., P. L. STRIPLIN, AND D. TSUKADA. 1980. Effects of screen size and replication on the Infaunal Trophic Index. South. Calif. Coastal Water Res. Proj. Biennial Rep. 1979–1980: 123–130.

ZAHL, S. 1977. Jackknifing an index of diversity. Ecology 58: 907–913.

Primary Production in a Tidally Energetic Fjord: Evidence of Seasonal and Inter-annual Tidal Forcing

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Kessler, T. A., and T. R. Parsons. 1989. Primary production in a tidally energetic fjord: evidence of seasonal and inter-annual tidal forcing. Can. J. Fish. Aquat. Sci. 46: 2166–2172.

A long term data set collected from a tidally energetic sill fjord was analyzed for its statistical relationships between primary production indices and several represented environmental variables. The analysis identified variance and covariance structure in these variables implicating changes in water column clarity, in inter-annual variability of phytoplankton carbon uptake rate, and the static stability of basin surface water in phytoplankton standing stock. The biomass–stability relationship was found to be seasonally dependent, with biomass positively correlated with stability in the summer and negatively correlated in the spring/fall, and restricted to waters under the direct mixing influence of the tidal inflow jet. These statistical patterns are discussed in terms of a possible control of primary production by seasonal and inter-annual variations in tidal inflow buoyancy.

On a analysé un ensemble de données recueillies sur une longue période dans un fjord-seuil soumis à une dynamique de marée afin de dégager les relations statistiques entre les indices de production primaire et plusieurs variables du milieu. L'analyse a permis de déterminer qu'il existe une structure de variance et de co-variance de ces variables qui implique des changements de limpidité de la colonne d'eau en fonction de la variabilité interannuelle du taux d'absorption du carbone phytoplanctonique, et la stabilité statique de l'eau superficielle du bassin en fonction de la biomasse phytoplanctonique. Il a été constaté que la relation de la biomasse à la stabilité varie avec les saisons, soit qu'il existe une corrélation positive entre la biomasse et la stabilité l'été, et une corrélation négative au printemps et à l'automne; enfin, elle est restreinte à l'eau soumise à l'influence directe du mélange causé par le courant-jet du jusant. Ces fluctuations statistiques sont envisagées dans la perspective d'un possible ajustement de la production primaire par des variations saisonnières et interannuelles de flottabilité de la masse d'eau apportée par le jusant.

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The general circulation in the Holberg-Rupert basin on Vancouver Island (Fig. 1) is dominated by an energetic tidal jet through the Quatsino Narrows entrance channel. The inflow characteristics vary seasonally owing to changes in local run-off and coastal upwelling conditions. During the winter when precipitation rate along the coast is maximal and the coastal wind field inhibits upwelling, the inflow is generally more buoyant than the resident basin water, while by midsummer the constant removal of basin salt by the less saline wintertime buoyant flow and a seasonal increase in upwelling result in a negatively buoyant inflow that typically sinks to the bottom of the basin within ≈2 km of the entrance (Stucchi and Farmer 1976). It has also been suggested that more rapid fluctuations in these run-off and upwelling conditions (i.e. on the order of days) can also affect the relative buoyancy of the tidal inflow (Stucchi 1985).

Fluctuations in jet penetration depth could be an important driving force of primary production. Since the jet entrains basin water, a change in depth, and hence a change in the location of the entrainment interface, could significantly alter field nutrient and phytoplankton entrainment fluxes with respect to the light field. Also, sufficiently negatively buoyant inflows have been linked with bottom sediment resuspension (Drinkwater and Osborn 1975; Stucchi and Farmer 1976) and so the basin light field per se may be directly affected as well.

While there is no substitute for a carefully designed field study to quantify any such connection between basin primary production and tidal inflow characteristics, some preliminary insight is possible from a long-term environmental monitoring data set that has been compiled for Holberg Inlet and surrounding waters. The data were collected by Island Copper Mine (ICM) as part of its British Columbia government permit to operate an open pit mine on the north shore of Rupert Inlet. Notably, the data set contains a long time series (i.e. 1976–82) of the vertical distribution of autotrophic carbon uptake rate and phytoplankton biomass at a series of standard stations in Holberg and Rupert Inlets and in Quatsino Sound. Statistical relationships between these biological variables and coincidental environmental parameter measurements (including derived indices of basin water column stability) will be presented and interpreted in terms of possible tidal inflow control of primary production.

Methods

The sampling regime consisted of six standard stations (Fig. 1) occupied once monthly from April to October. The variables measured were chlorophyl *a*, [Chl*a*], ^{14}C uptake rate, temperature, and salinity at the depths 1, 3, 5, 8 (or 10), 10 (or 15),

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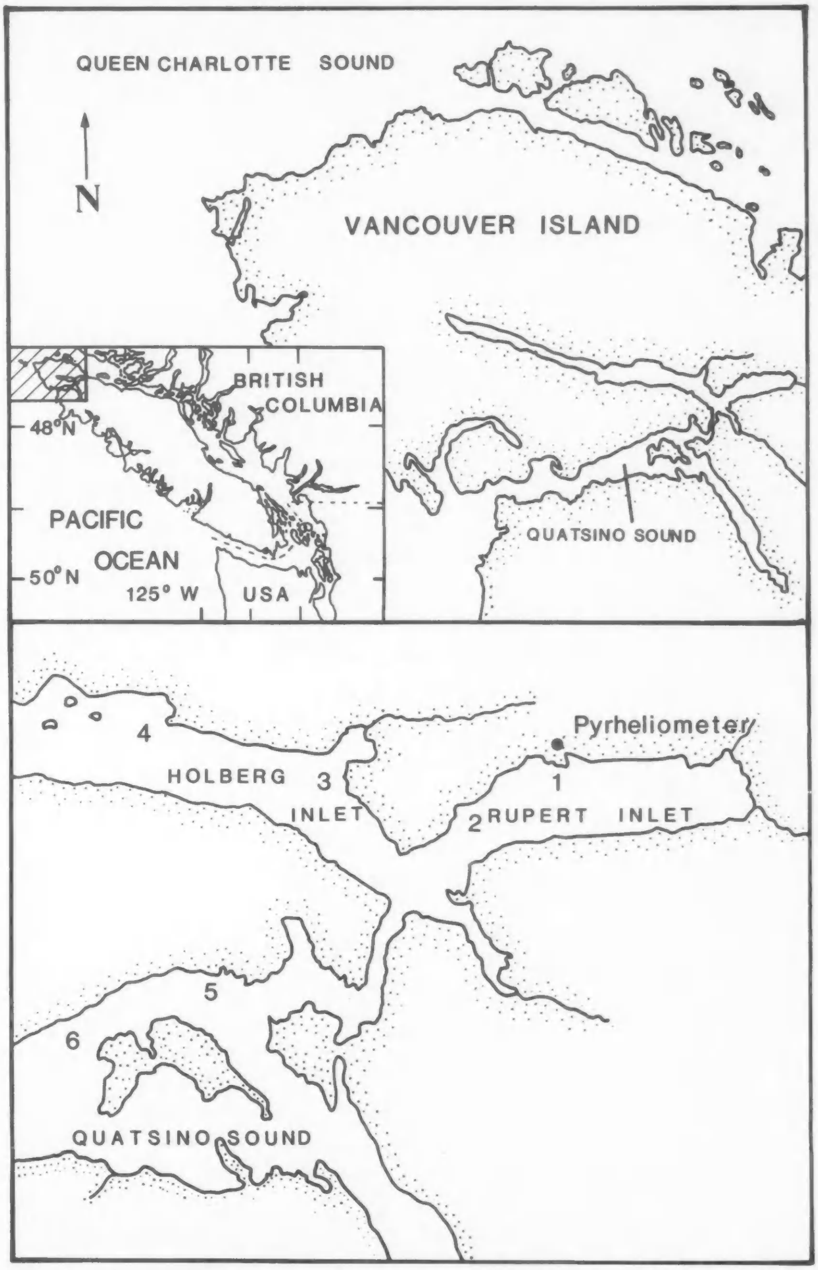
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HOLBERG
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INLET
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QUATSINO SOUND

FIG. 1. Location map of Holberg-Rupert basin Vancouver Island British Columbia (top) and data set standard station locations (bottom). Numbers denote station number.

and 20 m. In addition to these discrete profiles, coincident surface irradiance and, less consistently, water column light attenuance were also measured. Water samples were collected with 5-L Niskin samplers equipped with replicate reversing thermometers for temperature determination. Salinity was determined by titration according to Strickland and Parsons (1972). A 1-L subsample was vacuum filtered onto cellulose acetate/nitrate filters using $MgCO_3$ buffer and stored frozen over desiccant (maximum ≈2 wk) until they were analyzed for extractable chlorophyll. The spectrophotometric pigment determination followed the general procedure of Strickland and Parsons (1972). Carbon uptake rate was estimated from in situ incubations using replicate 125-mL Pyrex jars spiked with 5 μCi $NaH^{14}CO_3$. The ≈4-h incubations were performed between 10:00 and 16:00 local time. Activity in the particulate fraction was measured in Aquasol, with the calculation adjusted for dark uptake using counts from unreplicated in situ dark bottle incubations at 1, 3, 5, and 20 m depth. Incident broadband irradiance was measured with a Belford pyrheliometer located on the shore of Rupert Inlet (Fig. 1), while diffuse (photosynthetically active) light attenuance was measured using a cosine corrected in situ light meter employing a concurrent deck sensor.

A number of quantities were derived from this basic ICM data set to aid in the statistical analysis. Depth specific productivity $P(z)$ was obtained by normalizing the reported carbon uptake rate to extracted Chl*a* specific biomass $B(z)$. A depth integrated productivity index P^I was calculated by normalizing 0–15 m depth integrations of primary production to 0–15 m depth integrations of Chl*a* content B^S. These depth specific and depth integrated Chl*a* specific production rates were also normalized to the ^{14}C incubation averaged incident irradiance (I_0) to obtain a depth specific and depth integrated efficiency index (i.e. $Q(z)$ and Q^I, respectively). A mean near surface temperature T and light attenuance coefficient γ were also calculated from 0–5 m depth averages of the ICM data. The raw temperature and salinity data were also used to calculate a water column static stability parameter $E = 1/\rho(\delta\sigma_t/\delta z)$ (Pond and Pickard 1978, p. 27), which in the absence of more direct information provided an index of vertical mixing conditions in the surface layer. It was calculated both by finite differencing over the 0–5 m (E_T) and 5–25 m (E_B) depth intervals. Under the assumption that the 5 m depth stratum demarcates the surface (wind mixed) layer above the pycnocline (Pickard 1963), the two stability parameters allowed indirect inference to be drawn about the relative contribution of wind versus tidally generated mixing.

The variance structure of some variables was determined using a random design analysis of variance (Sokal and Rohlf 1981, p. 327). An assumption in any analysis of variance is that the underlying population variances for each of the data groupings are equal. A preliminary analysis of the data set indicated that this condition was not satisfied. Nevertheless, the raw data were left untransformed since analysis of variance techniques are also relatively robust in terms of this as well as other population distribution requirements. By comparison, the data transformations usually performed to ameliorate violations can seriously bias estimates of variance components attributable to interactions between data groupings (Lindman 1974). As will be shown below, these interactive effects dominate the variance structure. On the other hand, in the application used here, analysis of variance is particularly sensitive to data interdependence such as autocorrelation between successive sampling times (Lewis 1978). Temporal autocorrelation was not estimated for the data set. However, the pertinent rate constants (i.e. local phytoplankton growth rate, tide, and wind generated eddy diffusivities and advection velocities) can be expected to have characteristic time scales much shorter than the ≈30-d sampling interval underlying the data set. Hence autocorrelation was assumed to be unimportant.

The algorithm used in the analysis of variance (i.e. BMDP:8V, BMDP 1979, p. 598) requires a balanced data matrix. Thus in cases where one or two cells were empty, the iterative technique of Li (1964, p. 228) was used to approximate the missing elements. Otherwise, unbalanced data groupings were removed.

Both a parametric (partial correlation) and a nonparametric (Kendall's tau-b of concordance) statistic were used to calculate the co-variation between the variables. The nonparametric statistic provided a robust preliminary indication of relationships to be further explored using the more flexible parametric approach. The main difference between the two statistics aside from differing assumptions about data distribution is that Kendall's tau-b amounts to a simple correlation of ranks (Sokal and Rohlf 1981, p. 601) that does not differentiate between direct and indirect effects. By comparison, the partial correlation can be viewed as representing the simple (product–moment) correlation between the residuals from separate linear least-squares fits of the dependent variables against one or more common covariates. This linear detrending allows the unique co-variation between the dependent variables to be estimated (Sokal and Rohlf 1981, p. 656).

The tau-b statistic is robust in that it makes no assumptions about the underlying statistical distribution of the data. However, it is comparatively weak in rejecting a false null hypothesis (Zar 1974, p. 109). To compensate for this, tau-b significance, calculated according to Sokal and Rohlf (1981, p. 606), was tested at the $p \leq 0.10$ level.

To meet population distribution requirements of the partial correlation coefficient, the data were first normalized using the log–log regression procedure described by Elliott (1977, p. 73), with the D statistic used to test for distribution (Zar 1974, p. 83). The significance of correlations was determined by a t-test using the standard error $\sqrt{(1-r)/(n-m-2)}$, where r is the partial correlation, n is the sample size and m the number of fixed variables.

Results

Light attenuance (γ) has the most clearly defined variance structure of those variables evaluated. It is dominated by the year sampling effect, but is also characterized by year–station (y–s) and year-month (y–m) sampling effect interactions. All these components are statistically significant (Table 1). Since the highest order interaction represents the error term in a random design, the variance contributed by the three-way (year–station–month) interaction could not be determined.

The only clear variance component for the biological variables is the statistically significant y–m interaction. Some spatial–temporal variance structure (i.e. month–station) is also evident in the biomass normalized carbon uptake at 1 and 3 m (i.e. $P(1)$ and $P(3)$, respectively). However, this disappears when $P(z)$ is normalized to I_0 (i.e. $Q(z)$, Table 1), indicating that it is probably a reflection of asynoptic sampling and day to day variations in I_0 (ICM 1979).

For the co-variation analysis, the data were divided into a basin (stations 1–4) and Quatsino Sound (station 5 and 6) subset (Fig. 1). The underlying rationale is that mixing in Quatsino Sound would be relatively unaffected by the buoyancy of the tidal jet and hence this subdivision allowed a comparison of statistical relationships in the presence and absence of the putative entrainment flux control of primary production. Because of this contrast, it is in theory possible to separate this direct coupling from that mediated by changes in light field (i.e. from bottom sediment re-suspension), since the latter effect is geographically wide spread (ICM 1979). The absence of a spatial variance component for γ is consistent with this point (Table 1).

The tau-b matrix for the biological and environmental variables is presented in Table 2. Referring only to the co-variations between the dependent biological variables and the environmental indices: in the case of the Quatsino Sound subset, the statistically significant co-variations (i.e. $P \leq 0.10$) are the anticoncordance between integral biomass (B^S) and year and the

TABLE 1. The year, month, and station variance components in per cent for biomass ($B(z)$ and B^s), productivity ($P(z)$ and P^I), efficiency ($Q(z)$ and Q^I), and light attendance (γ). Asterisk denotes components statistically greater than zero ($P \leq 0.05$), while dash indicates the associated F-ratio<1. Depth in metres indicated in parentheses.

Source	γ	$B(1)$	$B(3)$	$B(5)$	B^s	$P(1)$	$P(3)$	$P(5)$	P^I	$Q(1)$	$Q(3)$	$Q(5)$	Q^I
Year (y)	28.4*	4.9	0.4	2.6	2.4	4.9	7.6	9.7	6.8	7.4	4.3	12	1.5
Station (s)	—	2.6	2.6	2.6	1.9	—	—	—	—	—	0.8	0.7	—
Month (m)	—	8.8	11	12	20	6.3	5.9	9.6	2.4	—	—	—	—
y–s	9.8*	—	—	—	0.5	—	3.9*	1.6	—	—	—	1.7	—
y–m	14*	18*	32*	38*	33	26*	29*	28*	20*	37*	42*	25*	24*
s–m	4.0	—	—	2.4	0.8	11*	12*	2.6	3.5	2.5	—	—	—

TABLE 2. Kendiall tau-b matrix for the basin and QS subsets of the ICM data set. In addition to the production indces P^I and B^S and the sampling variables month (mo), station (stn), year (yr), the array includes the environmental variables: 0–5 m depth averaged ambient temperature T, the incubation averaged incident irradiance I_0, and the static stability parameters E_T and E_B. Asterisk indicates significant concordance ($P \leq 0.10$).

	B^S	P^I	T	I_0	E_B	E_T
Holberg-Rupert basin ($n=165$)						
P^I	−0.052					
T	0.037	0.019				
I_0	0.235*	0.234*	0.020			
E_B	−0.015	−0.109*	−0.065	−0.053		
E_T	−0.223*	−0.119*	−0.013	−0.126*	0.354*	
mo	−0.136*	−0.106*	0.500*	−0.148*	−0.222*	−0.065
stn	−0.071	−0.003	−0.061	0.045	−0.027	−0.049
yr	−0.161*	0.063	−0.037	−0.184*	0.040	0.067
Quatsino Sound ($n=77$)						
P^I	−0.107					
T	0.104	−0.008				
I_0	0.061	0.136*	−0.016			
E_B	−0.009	−0.087	−0.008	0.012		
E_T	−0.038	−0.049	0.152*	−0.019	0.323*	
mo	−0.014	−0.070	0.608*	0.008	−0.134*	0.108
stn	0.112	0.041	0.149*	−0.089	0.318*	0.113
yr	−0.220*	0.077	−0.042	−0.199*	−0.060	−0.035

concordance between irradiance (I_0) and integral productivity (P^I). For the basin by contrast, B^S and P^I are both anti-concordant with at least one of the stability parameters as well as with month and are both concordant with I_0, while B^S is additionally anti-concordant with year.

Since I_0, E_B, B^S, and P^I all show anti-concordance with month (Table 2), it is not clear what among these co-variations can be attributed to direct interaction as opposed to co-variation with this common covariate. The same holds for B^S and I_0 since both are also concordant with year. Since the objective is to identify any direct relationships between environmental and biological variables, these sampling effects per se must be removed. While this is simply achieved by means of partial correlation coefficients, the associated linear detrending of the month effect is probably not the best approach owing to its nominal cyclical seasonality. Accordingly, the correlation matrix was linearly detrended only in terms of year using partial correlations, while the month effect was effectively removed by calculating separate correlation matrices for the data subdivided by month.

In the case of the Quatsino Sound subset, biomass (i.e. B^S and $B(z)$) remains generally uncorrelated with either of the stability parameters. In the basin by contrast, biomass is correlated with at least one stability index, with E_B by far the better predictor though E_T is effective in the spring/fall as well. There is also an apparent switch in the sign of the B–E_B correlation, being mostly positive in midsummer and negative in the spring/fall (Table 3).

Data subset differences are less in the case of P^I and $P(z)$ partial correlations. Here, I_0 is generally the best predictor in both data groups and is always positively correlated. Of the two stability parameters, E_T (in contrast to E_B for biomass) is the better predictor of productivity for the basin, while for Quatsino Sound, E_T and E_B are about equally strongly correlated with productivity. Unlike biomass, there is no indication of any seasonal sign dependence, with E_T and E_B generally negatively correlated with P^I and $P(z)$ in both the basin and Quatsino Sound.

For both data groups, I_0 is often strongly correlated with E_T and E_B (not presented). However, this does not confound the described interactions since partial correlations fixed relative to I_0 do not significantly alter the picture (i.e. note the parenthetical coefficients in Table 3).

Discussion

The variance structure for the biological variables is dominated by a temporal sampling effect (i.e. y–m) interaction. The

TABLE 3. Partial correlations (with year and station fixed) between biomass ($B(z)$ and B^S) and productivity ($P(z)$ and P^I) and, irradiance (I_0) and water column stability (E_T and E_B). The parenthetical coefficients calculated with I_0 fixed as well. Dash indicates a nonsignificant correlation ($P>0.05$). Rows with no significant coefficients have been deleted. Integers in parentheses denote depth in metres.

	April	May	June	July	August	September	October
Holberg-Rupert basin							
E_T							
$B(1)$	−0.461	—	—	—	—	—	—
$B(3)$	−0.618	—	—	—	—	—	—
$B(5)$	−0.641	—	—	—	—	—	−0.603
B^S	−0.613	—	—	—	—	—	−0.550
$P(1)$	—	−0.568(—)	—	—	—	0.456	−0.627
$P(3)$	—	−0.610	—	−0.474	—	—	−0.583
$P(5)$	—	−0.562(—)	—	—	—	—	—
P^I	—	—	—	—	—	0.568	(−0.495)
E_B							
$B(1)$	—	—	0.620	0.615	—	—	−0.531
$B(3)$	—	−0.509	0.583	0.508	—	—	−0.449
$B(5)$	—	−0.501	0.399	—	—	—	−0.429
B^S	—	−0.424	0.671	0.507	—	—	—
$P(5)$	—	—	—	—	(−0.426)	—	−0.562
P^I	—	—	—	—	—	—	−0.571
I_0							
$P(1)$	—	0.590	—	0.531	—	—	—
$P(3)$	—	0.589	—	0.465	—	—	—
$P(5)$	—	0.632	0.589	—	0.406	—	—
P^I	—	0.593	—	—	—	—	0.429
Quatsino Sound							
E_T							
$P(1)$	—	—	−0.657(—)	—	—	—	—
$P(5)$	—	—	—	—	—	−0.641(—)	—
P^I	—	—	—	−0.802	—	—	—
E_B							
B^S	—	—	—	—	—	—	−0.749
$P(1)$	—	—	—	—	0.687	—	—
P^I	—	—	—	−0.820(—)	—	—	—
I_0							
$P(1)$	—	—	—	—	—	—	0.694
$P(3)$	0.580	—	—	0.751	—	—	0.880
$P(5)$	0.970	—	—	0.925	—	—	—
P^I	—	—	0.649	0.780	—	—	—

lack of purely seasonal or inter-annual structure does not imply its absence (otherwise the associated interaction would also be absent), but rather occurs when this structure is short-lived compared with the sampling interval (Lewis and Platt 1982). This property of the ICM data set is consistent with the underlying sampling protocol and established theory on turbulent dispersion of phytoplankton. That is, a critical length scale for phytoplankton biomass can be defined by $\pi(K_h/\mu)^{1/2}$, where K_h is horizontal diffusivity and μ the phytoplankton specific growth rate. This defines the horizontal patch size for which growth and dispersive effects are approximately equal, allowing patches to remain near steady state, and hence represents a region where spatial heterogeneity is maximal (Okubo 1978; Legendre and Demers 1984). In other words, on shorter length scales mixing effects are the primary determinants of phytoplankton distribution regardless of in situ growth rate, while on longer scales it is phytoplankton in situ growth which is more important. An empirical estimate of the upper and lower limit can be determined from the variance spectrum (i.e. variance as a function of fluctuation frequency) or horizontal in vivo fluorescence profiles. These generally indicate a transition from $-5/3$ to -1 in the slope of the spectrum (i.e. indicating a transition from conservative to nonconservative tracer) at length scales ranging from ≈1–10 km (Denman and Platt 1975; Fasham and Pugh 1976; Lekan and Wilson 1978).

At least for instances when dispersion comes to dominate over in situ growth, a crude time scale can be associated with the critical length scale domain. For example, Okubo (1971) observed a relationship between the diffusion time scale and the horizontal patch size of a conservative tracer, with the size expressed as a standard deviation $\sigma = 0.6 \cdot t^{1.17}$. Substituting the lower (1 km) and upper (10 km) limit for the slope transition in the variance spectrum and solving for t gives diffusion times from a point source and hence a characteristic time scale of persistence within this spatial domain of 7 d. This is a factor of four less than the ≈30-d sampling interval. Hence, at least for times when phytoplankton biomass approximates a conservative tracer, the temporal resolution of the sampling protocol is coarse compared with the time scale of change in phytoplankton distribution. A similar argument holds for the productivity variables given their indirect spatial dependence (i.e. their dependence on nutrient, light, and normalizing biomass distributions).

The contrast in time scales does not prevent statistical inferences being drawn from the data variance structure. The relative dominance of the y–m sampling effect interaction (as compared with y–s or m–s) for the biological variables can be interpreted as meaning that the inter-annual difference in the inter-month contrast is large compared with the inter-annual difference in the spatial contrast. In other words, the inter-annual factor detected in the variance structure is statistically more dependent on season than location. This would appear to obviate direct tidal mixing (i.e. the inflow tidal jet entrainment regime) as an important process underlying this y–m interaction since, to be consistent, the latter would also manifest as a spatial effect (i.e. affecting the basin but not Quatsino Sound). Furthermore, the dominant inter-annual variance component in attenuance suggests that the inter-annual phytoplankton variance structure underlying the y–m interaction may be coupled to water column clarity.

The P–I_0 partial correlation, in the absence of any indication that it is confounded by other interactions, can be taken as evidence that growth is generally light limited. If this is the case, then a plausible explanation for the apparent negative P-stability correlation for the basin is that stability is itself positively correlated with light attenuance. As mentioned at the outset, the deepwater sediments near Quatsino Narrows are known to undergo vigorous resuspension in response to the scouring action of the tidal jet, particularly when it is negatively buoyant. Hence, a decrease in basin water column density during the summer (reflected as an increase in stability), could on the one hand, result in a sufficient increase in the relative density of the inflow to cause increased sediment resuspension and water column light attenuance from bottom scouring. On the other hand, the negative P-stability correlation for Quatsino Sound appears to argue against this since a denser inflow should be associated with reduced stability in Quatsino Sound. However, this may not be seriously constraining since stability is normally lower in Quatsino Sound near the surface region than in the basin: monthly mean E_T ranged (in units of 10^6 per metre) from 135 in August to 800 in September for the basin and from 36 in August to 209 in September for Quatsino Sound. This difference could conceivably decouple the relative buoyancy of the tidal inflow from fluctuations in Quatsino Sound water stability.

There is additional historical data supporting a connection between bottom sediment resuspension and inter-annual fluctuation in attenuance. Local bathymetry data (ICM 1983) shows pronounced inter-annual variability in basin sediment resuspension adjacent to Quatsino Narrows, with a net loss of bottom sediments occurring between 1976–80 and a net gain between 1980–82. The year to year fluctuation in annual mean γ is coherent with this pattern of sediment resuspension inferred from the bathymetry data. The annual mean light attenuance in the basin increased from $0.35 \cdot m^{-1}$ in 1976 to $0.50 \cdot m^{-1}$ in 1979 (i.e. during the period of net bottom sediments loss) and then declined again to $0.30 \cdot m^{-1}$ in 1982 (i.e. coinciding with the period of net deposition, ICM 1983).

The clearest seasonal signal in the analysis is the sign reversal in the B-E_B partial correlation for the basin. Coupling between water column stability and plant biomass concentrations can be expected to include both the indirect effect of stability on in situ growing conditions partially addressed above, and the direct effect of stability on biomass dispersion processes. The seasonal sign reversal in B-E_B coincides with the nominal transition from predominantly negatively buoyant to positively buoyant inflow (Stucchi and Farmer 1976). Furthermore, the opposite sign of the B-E_B and P-stability co-variation in the midsummer basin implies that whatever the mechanism underlying the P-stability relationship, the apparent stability control of direct dispersion effects is the dominant process during this time. If this were not so, B-E_B would have the same sign as P-stability. This allows the inference that the seasonal signal in B-stability (i.e. sign reversal) is probably not simply an additional manifestation of light attenuance limited productivity, but rather is directly related to some stability effect on standing stock accumulation. Furthermore, since stability below the pycnocline (i.e. E_B) appears to be more closely related to this biomass dispersion effect than stability in the (wind mixed) surface layer (i.e. E_T), this dispersion effect is more likely a tidal as opposed to wind generated process. Finally, going one step further, since the greater predictive power of E_B is restricted to the basin, it would be consistent to suggest that the seasonality of the tidal dispersion effect is control by inflow relative buoyancy.

In summary, statistical features of the ICM dataset lend support to the suggestion that the tidal inflow regime is a controlling factor of primary production in the basin. This control appears to have both an inter-annual and seasonal character. While both features appear to be dependent upon changes in the relative buoyancy of the tidal inflow, the inference is made that inter-annual control is mediated through changes in bottom sediment tidal resuspension and its affect on light limited phytoplankton growth, while seasonal control arises through variations in the rate of standing stock dispersion.

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References

BMDP (Biomedical Computer Programs P-series) 1979. Health Sciences Computing Facility, Dept. Biomathematics, Univ. of Cal. University of California Press, Los Angeles 880 p.

Denman, K. L., and T. Platt. 1975. Coherence in the horizontal distribution of phytoplankton and temperature in the upper ocean. Proceedings of the 6th liège Colloquium in Ocean Hydrodynamics, Mémoires de la Société Royale des Sciences de liège 6 ser 7: 19–30.

Drinkwater, K. F., and T. R. Osborn. 1975. The role of tidal mixing in Rupert and Holberg Inlets, Vancouver Island. Limnol. Oceanogr. 20: 518–529.

Elliott, J. M. 1977. Some methods for the statistical analysis of samples of benthic invertebrates. Freshwater Biol. Assoc. Sci. Pub. No. 25, 2nd ed. 157 p.

Fasham, M. J. R., and P. R. Pugh. 1976. Observations on the horizontal coherence of chlorophyll *a* and temperature. Deep Sea Res. 23: 527–538.

ICM (Island Copper Mine). 1983. Island Copper Mine 1982 Annual Environmental Impact Assessment Report. Vol. 1, Island Copper Mine, Port Hardy British Columbia, 106 p.

1979. Island Copper Mine 1978 Annual Environmental Impact Assessment Report. Vol. 1, Island Copper Mine, Port Hardy British Columbia, 99 p.

Lekan, J. F., and R. E. Wilson. 1978. Spatial variability of phytoplankton biomass in the surface waters of Long Island. Est. and Coast. Mar. Sci. 6: 239–251.

Legendre, L., and S. Demers. 1984. Towards dynamic biological oceanography and limnology. Can. J. Fish. Aquat. Sci. 41: 2–19.

Lewis, M. R., and T. Platt. 1982. Scales of variability in estuarine ecosystems. p. 3–20. *In* Estuarine comparisons, V. S. Kennedy [ed.] Proceedings of the 6th Biennial Int. Est. Research Conference. Gleneden Beach, Oregon, Nov. 1981. Academic Press.

Lewis W. M., Jr. 1978. Comparison of temporal and spatial variation in the zooplankton of a lake by means of variance components. Ecol. 59: 661–671.

Li, C. C. 1964. Introduction to experimental statistics. McGraw Hill, New York, NY. 460 p.

Lindman, H. R. 1974. Analysis of variance in complex experimental designs. W. H. Freeman and Co., San Francisco, CA. 352 p.

Okubo, A. 1978. Horizontal dispersion and critical scales for phytoplankton patches, p. 21–42. *In* Spatial patterns in plankton communities. J. H. Steele [ed.] Plenum Press New York, NY.

1971. Ocean diffusion diagrams. Deep Sea Res. 18: 789–802.

Pickard, G. L. 1963. Oceanographic characteristics of inlets of Vancouver Island, British Columbia. J. Fish. Res. Board Can. 20: 1109–1144.

Pond, S., and G. L. Pickard. 1978. Introductory dynamic oceanography. Pergamon Press, New York, NY. 241 p.

Sokal, R. R., and F. J. Rohlf. 1981. Biometry. W. H. Freeman and Co., San Francisco, CA. 859 p.

Strickland, J. D. H., and T. R. Parsons. 1972. A practical handbook of sea water analysis. J. Fish. Res. Board Can. Bull. No. 167. 311 p.

Stucchi, D. 1985. The tidal jet in Rupert-Holberg Inlet, B.C. Atmos. Ocean. 23: 118–136.

Stucchi, D., and D. M. Farmer. 1976. Deep-water exchange in Rupert-Holberg Inlets. Inst. Ocean Sci., Patricia Bay. Pacific Mar. Sci. Rep. 76–10.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall Inc. London. 620 p.

Primary Production in a Tidally Energetic Fjord: Environmental Forcing of Vertical Phytoplankton Distribution

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Kessler, T. A., and T. R. Parsons. 1989. Primary production in a tidally energetic fjord: environmental forcing of vertical phytoplankton distribution. Can. J. Fish. Aquat. Sci. 46: 2173–2183.

Time dependent changes in the vertical phytoplankton distribution, during two short-term intensive studies in a tidally energetic sill-fjord, were examined with the objective of identifying processes responsible for a seasonal dependence between biomass and stability in a 7-yr environmental data set for the region. Reconciling an apparent divergence between calculated chlorophyl a (Chla) and NO_3^- mass balances identified diapycnal mixing to be an important term in the NO_3^- budget, but not in the Chla budget. The balancing mixing rate required was consistent with a wind stress origin and inconsistent with a tidal origin. This calculation, combined with evidence that mixing events in the surface layer were generally more closely correlated with the wind than the tide was used to support a hypothesis relating the seasonal biomass–stability relationship to seasonally dependent tidal mixing in the surface layer.

Au cours de deux courtes études intensives faites dans un fjord-seuil soumis à une dynamique de marée, les variations dans le temps de la distribution verticale du phytoplancton ont été examinées afin de mettre en évidence les processus à l'origine d'une dépendance saisonnière entre la biomasse et la stabilité qui est révélée par un ensemble de données écologiques recueillies sur 7 ans dans la région. La résolution d'une apparente divergence entre le bilan de masse calculé de la chlorophylle a et celui du NO_3^- a permis de montrer qu'un mélange diapycnal constitue un terme important du bilan du NO_3^-, mais non pas du bilan de la chlorophylle a. Le taux de mélange requis à l'équilibre correspondait bien à un stress éolien et ne correspondait pas un stress d'origine tidale. Ce calcul, lorsqu'il est combiné à des indices à l'effet que des évènements de mélange dans la couche superficielle d'eau sont généralement en plus étroite corrélation avec le vent qu'avec les marées, a servi à défendre l'hypothèse à l'effet qu'il existe une relation entre le rapport saisonnier de la biomasse à la stabilité et le mélange tidal selon les saisons dans la couche superficielle d'eau.

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Tidal exchange in the Holberg-Rupert basin, Vancouver Island features an inflow jet through the Quatsino Narrows entrance channel (Fig. 1). The manner in which this jet mixes with the basin water column is dependent upon its buoyancy. During the winter months when the jet is typically less dense than the basin, the inflow penetrates the basin interior by entraining water from above, while by midsummer the now more usually negatively buoyant flow descends to the bottom of the basin directly through gravitation (Stucchi and Farmer 1976). Higher frequency fluctuations in run-off and offshore upwelling conditions also impose shorter term variations onto this seasonal pattern (Stucchi 1985).

The analysis of a 7-yr environmental monitoring data set for the basin and surrounding waters (Kessler and Parsons 1989) indicated a seasonally dependent relationship between the basin water column static stability and phytoplankton standing stock (i.e. positive correlation in summer and negative correlation in spring/fall). Furthermore, this seasonality was apparently restricted to the basin proper and was more closely related to pycnocline stability below 5 m than above. This suggests that phytoplankton distribution in the basin may be dependent on the seasonal buoyancy properties of the tidal inflow.

Two short-term studies were carried out in 1982 to more closely examine the role of basin mixing in standing stock distribution. These studies were in May and August when, according to the historical data set, growth (i.e. carbon uptake rate) was consistently negatively correlated with stability, while biomass and stability, by comparison, were either negatively (May) or positively (August) correlated (Kessler and Parsons 1989).

A preliminary survey of Holberg Inlet over a 1-wk period in June 1981 (T. A. Kessler, unpubl. data) indicated the geographically widespread occurrence of a subsurface chlorophyll maximum (SCM) at ≈3m depth. Therefore, to rationalize the 1982 studies, it was hypothesized that the dynamics of vertical biomass distribution may be an important factor in the seasonality of a biomass–growth–mixing relationship. Hence these 1982 studies were directed at short term processes affecting vertical biomass distribution and how this could vary seasonally.

The design of the sampling protocol was based on two considerations. First, the practicable design was tailored to maximize the resolution of vertical biomass distribution (Cullen 1982). Second, since relative scaling of mixing and phytoplankton growth in a Holberg-Rupert basin size water mass argues that, over a time period of days, response to external forcing is spatially nonuniform in the horizontal (Lewis and Platt 1982), drift station sampling was used in place of fixed locations. A

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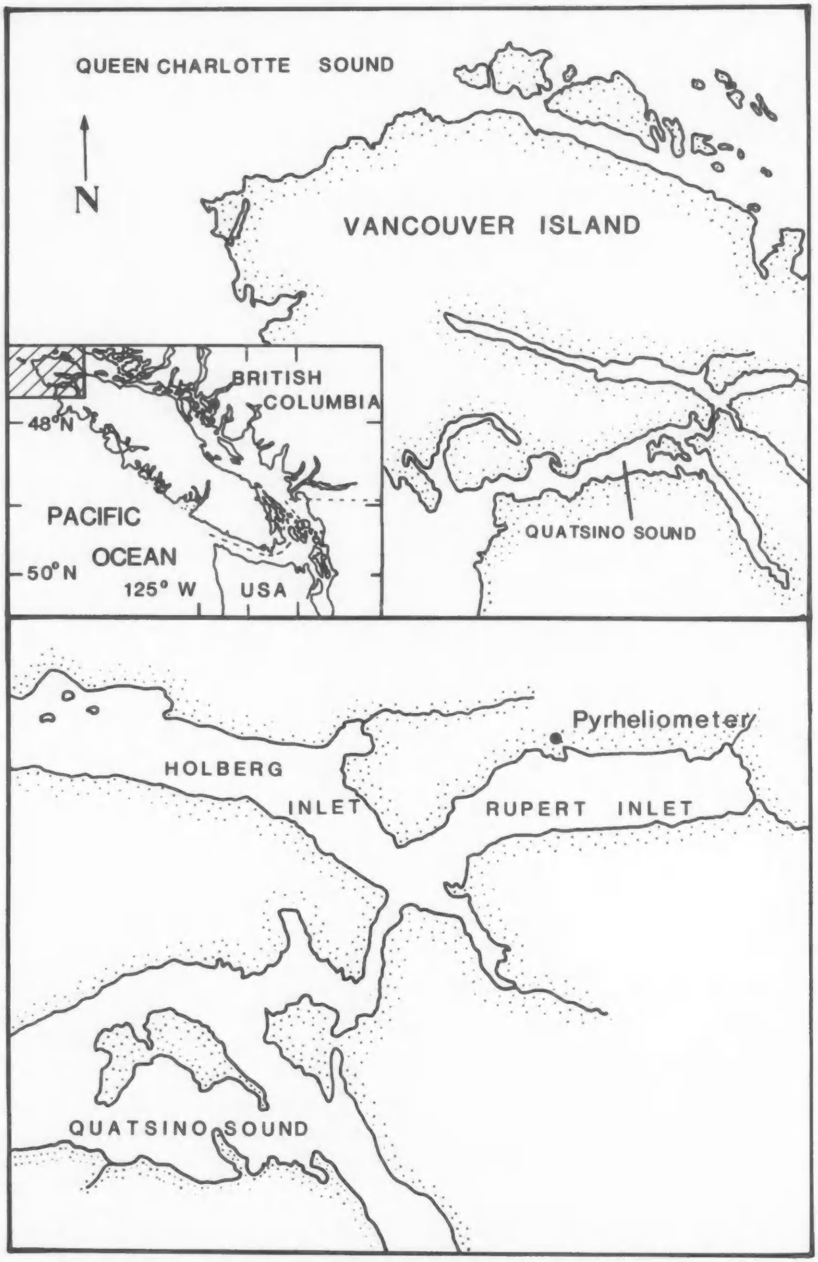
QUEEN CHARLOTTE SOUND
N
VANCOUVER ISLAND
BRITISH
COLUMBIA
48°N
PACIFIC
OCEAN
50°N
125° W
USA
QUATSINO SOUND
Pyrheliometer
HOLBERG
INLET
RUPERT INLET
QUATSINO SOUND

FIG. 1. Location map of Holberg-Rupert basin on Vancouver Island, British Columbia.

drift station time series would be relatively immune from the effects of this heterogeneous response. Similarly, while the Lagrangian reference frame did not allow horizontal flux terms to be ignored outright, contributions could at least be qualified on the basis of estimated vertical shear.

Methods

Sampling was confined to Holberg Inlet as it and Rupert Inlet are generally symmetrical with respect to Quatsino Narrows. The water parcel was marked with a radar reflector equipped drifter, described by Buckley (1977), with its 2-m deep sail placed at a mean depth of 3 m. The vertical distribution of chlorophyl *a* (Chl*a*), NO_3^-, ^{14}C uptake, microscope phytoplankton cell counts, salinity, temperature, and downwelling irradiance was measured, though not all measurements were carried out during all sampling times. The discrete analyses were performed on subsamples from a bulk 6-L sample collected using a Jabsco diaphragm bilge pump, with intake depth

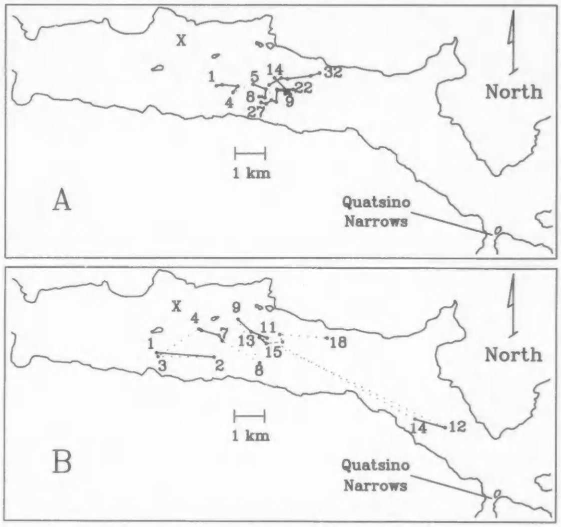

FIG. 2. Drift station drogue tracks for May (top) and August (bottom). Numbered points correspond to position numbers in Table 1. Solid lines connect continuously observed station drift, while dotted lines connect overnight interpolations. The letter *X* marks the wind anemometer location.

determined using a hydro-wire meter wheel. To ensure that no major features in vertical structure were inadvertently missed by the discrete sampling protocol, a Turner 111 fluorometer (connected to the pump outflow) was used to estimate chlorophyll distribution by in vivo fluorescence.

Chl*a* determinations were done on a 1-L subsample vacuum filtered (<15 mm Hg) onto either Whatman 984AH or GF/C (43 mm) glass-fiber filters. Filters were stored frozen over desiccant until analysis (i.e. usually ≈2 wk) following the spectrophotometric procedure of Strickland and Parsons (1972) and using the equations of Jeffrey and Humphrey (1975). Ambient [NO_3^-] was measured manually (Strickland and Parsons 1972) on refrigerated samples within 10 h of collection. ^{14}C-fixation rate was determined using 3–4 h (midday) in situ incubations of replicate 250-mL subsamples spiked with 5 μCi $NaH^{14}CO_3$. Incubation depths were usually at 2-m intervals from the surface to 20 m, decreasing to intervals of 1 m or less near the SCM. The ^{14}C uptake by the particulate fraction (i.e. retained on the acetate/nitrate membrane filters) was measured in Aquasol after rinsing the filters with a standard amount of filtered seawater. Incident irradiance was measured with a broadband sensor equipped LiCor LI185B radiometer located on ship deck. The sensor response was converted to PAR (i.e. 400–700 nm) using the conversion factor 0.37 (LiCor publication 8004-03). Total daily irradiance was obtained from a Belford broadband pyrheliometer located on the shore of Rupert Inlet and maintained by Island Copper Mine, Port Hardy British Columbia (Fig. 1). The LiCor conversion factor was also used to convert the pyrheliometer broadband irradiance to approximate PAR. Vertical profiles of downwelling irradiance were obtained either with a PAR quantum sensor equipped LI185B or a Biospherical Instruments MER1000 PAR integrating spectroradiometer. Wind velocity was measured with a propeller type anemometer (i.e. NOAA W102-P Skyvane 1) located midway up Holberg Inlet (Fig. 2) and positioned 5 m above mean sea level on an island measuring east–west ≈100 m long and that was no more than 3 m above mean sea level at its highest point. The nearest wind obstruction was located ≈1 km to the west. Surface current shear was estimated by attaching a surface floating InterOcean model 135 savonious rotor type current meter to the drift marker, with the profile measured relative to drogue depth as the meter was raised and lowered in discrete steps at ≈20-min intervals. Temperature and salinity profiles were determined in May using a Bissett-Berman STD (salinity-temperature-depth probe) and in August using an Autolab model 602 Wheatstone bridge temperature–salinity probe.

Results

The observed drogue tracks are presented in Fig. 2. Since position was determined only during daylight hours (i.e. during station sampling), the plotted tracks include overnight interpolations; which are represented as dotted lines. During May, continuous tracks were limited to 2–3 d in duration before the drogue would beach in shallow water. In total, one 3-d (henceforth referred to as M1) and two 2-d (M2 and M3) drift station time series were obtained. These stations were sampled daily. In August two drogues were deployed simultaneously, from which one 9-d and two 3-d drift station time series were obtained, though only results from the 9-d station (A1) will be presented. Drift stations in August were occupied no more frequently than every other day.

Drogue Tracks in relation to Wind and Tide

It was generally not possible to separate the effects of wind stress and tidal excursion on drogue displacement, since most of the drogue tracking coincided with periods when the wind was oriented essentially parallel to tidal flow. However, some discrimination is possible for two occasions: the southwestward M2 drogue track on May 16 was inconsistent with the predominant southerly wind and consistent with the flooding tide, while the westward M2 drogue track on May 17 was consistent with the predominant northeasterlies and inconsistent with the ebbing tide (see Fig. 2a and Table 1). Thus, in these two instances where wind stress and tidal flow were not parallel, currents in the 2–4 m depth stratum appeared to be of tidal origin for the flood tide and driven more by wind stress on the ebb tide.

Further information on the relationship between surface currents and varying tide and wind conditions can be estimated from the current shear profiles. Since the current meter used does not vector average, the calculated component current shears were evaluated only in terms of directional orientation. It should also be noted that these shear profiles were not strictly synoptic since the total profiling time of ≈100 min comprises a significant part of the semidiurnal tidal cycle. A common feature of the profiles is a distinct shear distributed between 1 and 5 m (Table 1). Its relatively constant orientation to wind direction suggests a wind stress origin. For example, during northwest winds on May 11, the current at 1 m relative to the eastward drifting drogue (Fig. 2a) was to the east and south, with no shear detectable below drogue depth. Similarly, during northwest winds on August 9, the current relative to the eastward drifting drogue (Fig. 2b) was to the east and south at 1 m and to the west and north at 5 m. Relating this overall pattern to the tidally dominated current at drogue depth on May 16 suggests that, during flood tide at least, near-surface shear appears to be distributed over the upper ≈2 m. The isothermal mixed surface layer on May 16 and 17 (Fig. 3) is also evidence

TABLE 1. Drift station sampling dates with position numbers corresponding to Fig. 2, along with sampling time tidal state (F = flood, E = ebb), mean wind speed and direction, and current speed at 1 m and vector components at 1 m (U1, V1) and 5 m (U5, V5) all relative to drogue depth. U is east–west coordinate and V is north-south coordinate. Dots and dashes denote below detection limit and no measurement taken, respectively.

Drift station	Date (time)	Position numbers	Tide	Wind ($m \cdot s^{-1}$)	Direction	Surface current ($cm \cdot s^{-1}$)	U1/U5	V1/V5
M1	May 11 (07:00–15:45)	1 to 4	E	1	NW	2.8	+/·	−/·
	May 12 (08:50–14:20)	5 to 8	E	3	NW	·	·/·	·/·
	May 13 (09:45–12:30)	9 to 14	E	1	NW	2.9	+/·	−/·
M2	May 16 (12:45–17:40)	22 to 27	F	10	S	10.2	−/−	+/−
	May 17 (12:15–16:40)	28 to 32	E	4	NE	4.8	−/−	−/+
A1	August 09 (08:00–12:30)	1 to 2	E	3	NW	7.3	+/−	−/+
	August 10 (14:40)	3	E	< 1	NW	—	—	—
	August 11 (09:14–14:10)	4 to 7	E	3	NW	4.3	+/−	+/·
	August 12 (09:45)	8	E	< 1	W	—	—	—
	August 13 (11:00–14:20)	9 to 11	E	5	NW	10.8	+/−	−/·
	August 17 (10:45–17:08)	16 to 17	E	< 1	NW	—	—	—

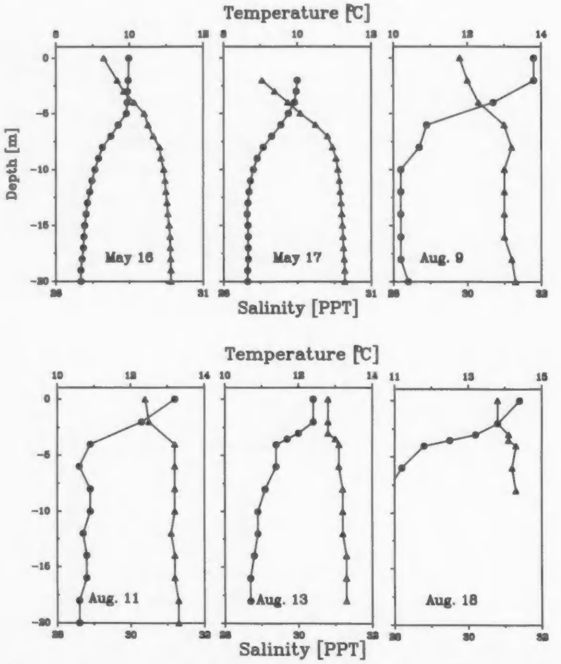

FIG. 3. Temperature (circles) and salinity (triangles) profiles for drift stations M2 (May 16, 17) and A1 (August 9, 11, and 13).

that wind speeds of $\approx 10\ m \cdot s^{-1}$ effectively mix the surface to 5 m. This was the only occasion in a total wind record of $\approx$100 d (discontinuously spanning May to August) when sustained wind speed exceeded 6 $m \cdot s^{-1}$. Hence, this sort of wind event appears to be rare for the basin in the summer, and by inference so is a 5 m mixed layer. However, since wind direction during this deep mixing was not optimally aligned with the basin, lower wind speeds may have similar consequences in mixing when oriented more favorably. Tidal state undoubtedly contributed as well.

Evolution of Vertical Phytoplankton Distribution

In the interests of brevity only stations M2 and A1 will be discussed as these stations showed the greatest variability in biomass distribution. Station M2 Chl*a* distribution changed from monotonically decreasing with depth on May 16 to a pronounced SCM on May 17 situated at 6 m, well above the estimated 1% light depth of $\approx$20 m. However, on both days, ^{14}C-uptake normalized to Chl*a* specific biomass (P^B) decreased with depth in an essentially monotonic fashion, while [NO_3^-] remained generally >4 μM, except at the SCM depth on May 17, where it declined to <1 μM (Fig. 4). The 0–4 m integrated Chl*a* increased by 6.3 $mgChla \cdot m^{-2}$ over the 2 d, while NO_3^- declined by 161 mg $NO_3^- \cdot m^{-2}$. By comparison, at 6 m Chl*a* increased by 19 $mgChla \cdot m^{-3}$ and NO_3^- declined by 145 mg $NO_3^- \cdot m^{-3}$. Assuming a N/Chl*a* of three, the NO_3^- disappearance is a mass equivalent factor of nine greater than the Chl*a* increase. This compares with only a factor of three difference at SCM depth.

There was some indication of a SCM at the start of A1 at $\approx$2 m, though the corresponding fluorescence profile declined monotonically (Fig. 5). Ambient [NO_3^-] was >4 μM and P^B declined persistently with depth (Fig. 6). By August 10, the fluorescence profiles clearly indicated a SCM at 3–4 m, confirmed as a Chl*a* feature near the base of the pycnocline on August 11. Again, this was located well above the 1% light level of $\approx$15 m. The August 11 near surface [NO_3^-] was reduced significantly from August 9, but was still >1 μM. The profile of P^B continued to decrease monotonically, though a secondary peak was evident at 2.5 m. The between replicate difference for the latter was relatively large, however. In both cases, the overall rate of decline in P^B with depth combined with the distribution in biomass resulted in the maximum in total carbon uptake occurring slightly shallower than the SCM. The extractable Chl*a* and in vivo fluorescence data give conflicting indications about the evolving biomass content of the SCM. In vivo fluorescence (in arbitrary units) increased from $\approx$35 on August 11 to $\approx$45 on August 13 (fluorometer gain remaining essentially constant), though on the basis of extracted [Chl*a*], SCM biomass had actually decreased by $\approx$50% during this period. While the SCM remained depth contant after August 11, the peak in total carbon uptake increased in depth, apparently in step with the descending peak in P^B. All peaks were situated at $\approx$3 m by August 13. The upper $\approx$2 m temperature (Fig. 3), [Chl*a*], and [NO_3^-] on August 13 showed indications that a shallow mixing event had occurred between August 11 and 13.

There was no further detailed sampling after August 13. However, on the basis of a STD and in vivo fluorescence profile

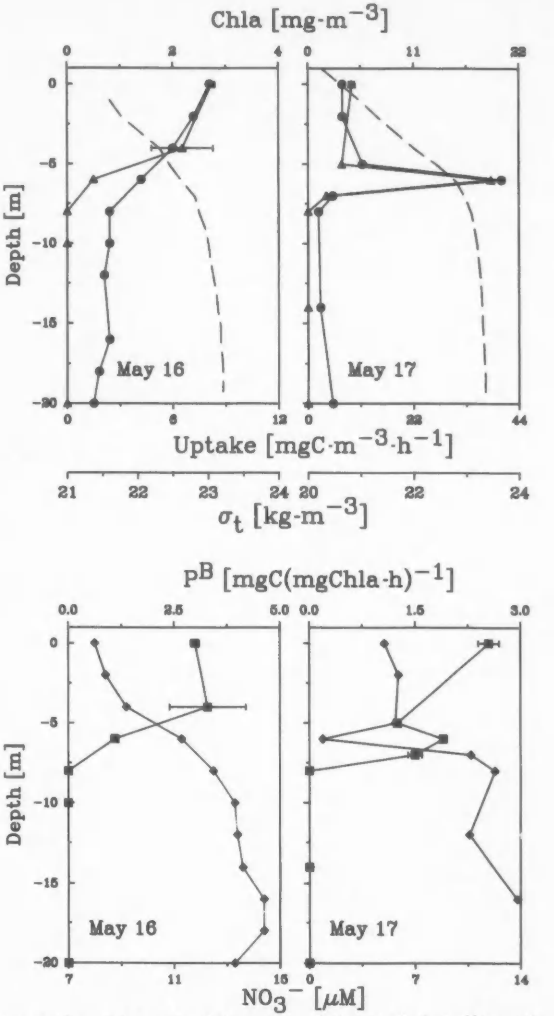

FIG. 4. Top, drift station M2 profiles of [Chl*a*] (circles), ^{14}C-uptake (triangles), and sigma-*t* (dashed line). Bottom, M2 profiles of Chl*a* normalized ^{14}C-uptake P^B (squares) and $[NO_3^-]$ (diamonds). Error bars for ^{14}C-uptake and P^B indicate standard error.

on August 17, the 3-m SCM at least, appeared to remain a constant feature through to August 17. Furthermore, the presence of this feature appeared to be unaffected by the large station drift after August 13 (Fig. 2b).

Phytoplankton Species Composition

There were no species enumeration samples collected at M2. However, samples obtained at 0 m on May 18 and 0 and 3 m on May 19 indicated two dominant species in the assemblage. The diatom *Thalassiosira decipiens* (cell volume ≈5200 μm^3) and an unidentified autotrophic cryptomonad (cell volume ≈700 μm^3) were present in the three samples, with *T. decipiens* the dominant species on a volume basis in two cases, ranging from 66% at the surface to 91% at 3 m on May 19. The cryptomonad was dominant at the surface on May 18, comprising 54% of the sample (compared with 4% for *T. decipiens*). The autotrophic ciliate *Mesodinium rubrum* occurred in trace amounts in all three samples.

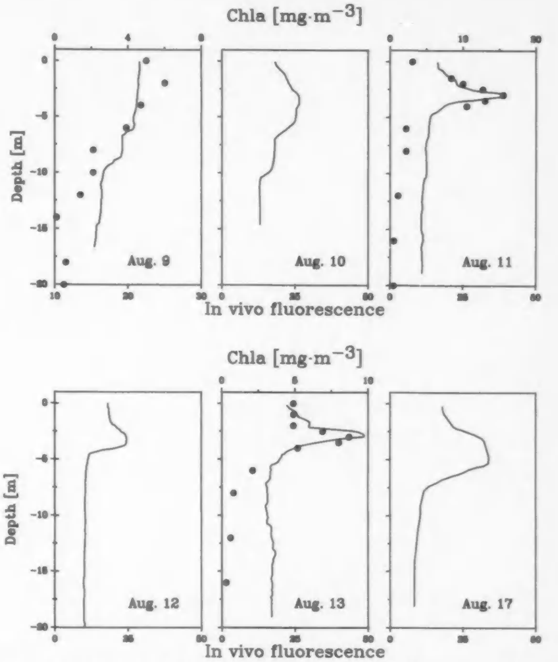

FIG. 5. Drift station A1 [Chl*a*] (circles) and in vivo fluorescence (line) profiles.

The relative abundance of the dominant autotrophs at A1 is presented in Table 2. The diatom *Thalassiosira polychorda* was dominant at the surface on August 9, with slightly smaller contributions by two autotrophic dinoflagellates, two poorly preserved autotrophic flagellates, and the autotrophic ciliate *Mesodinium rubrum*. The two flagellates were tentatively identified as a cryptophyte and the chrysophyte *Isochyrsis galbana* (F. J. R. Taylor, Department of Oceanography, University of British Columbia, pers. comm.). At 10 m, the assemblage was dominated by the chrysophyte.

On Aug. 11 the surface consisted equally of *Peridinium* sp. and *M. rubrum*. This shift in relative abundance compared with August 9 stemmed from a 10-fold decline in *T. polychorda* and five-fold increase in *M. rubrum*. At 2.5 m, corresponding to the upper limit of the SCM, the chrysophyte was dominant with secondary contributions from *Chaetoceros* spp. (cell volume = 1000 μm^3) and *M. rubrum*.

On August 13, the surface was dominated by the chrysophyte and *Chaetoceros* spp., with secondary contributions by *Peridinium* sp., *Dinophysis* sp., the cryptomonad, and *M. rubrum*. This shift in relative abundance was due to a two-fold reduction in *Peridinium* sp. and *M. rubrum*, a three-fold increase in the chrysophyte and a 40-fold increase in *Chaetoceros* spp. At 2.5 and 3 m, the assemblage was also dominated by the chrysophyte, with secondary contributions by *Peridinium* sp., *M. rubrum*, and *T. polychorda*. In this case the shift in relative abundance was due primarily to a five-fold reduction in *Chaetoceros* spp.

Thus, summarizing the A1 enumeration data, the distribution of the major phytoplankton species evolved from a mixed assemblage on August 9 to one dominated by autotrophic dino-

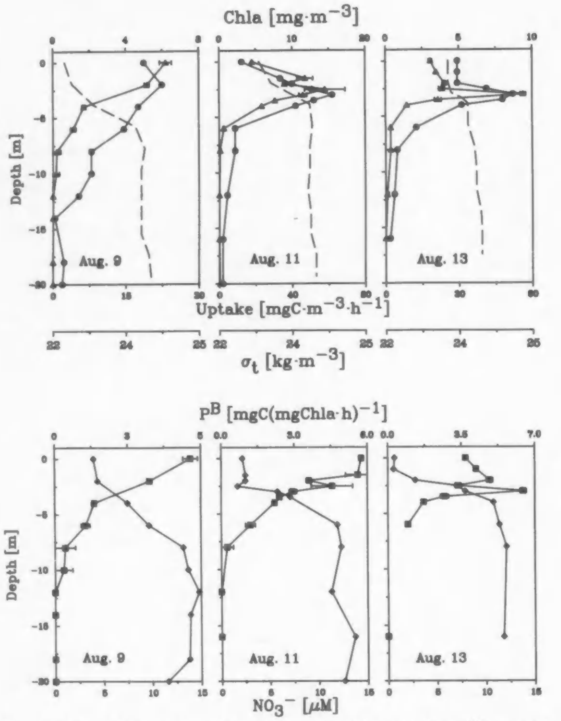

FIG. 6. Top, drift station A1 profiles of [Chl*a*] (circles), ^{14}C-uptake (triangles), and sigma-*t* (dashed line). Bottom, A1 profiles of Chl*a* normalized ^{14}C-uptake P^B (squares) and [NO_3^-] (diamonds). Error bars for ^{14}C-uptake and P^B indicate standard error.

flagellates and a ciliate at the surface and by diatoms and autotrophic flagellates at depth (i.e. the SCM) on August 11. This was followed by a transition to a diatom and flagellate dominated surface assemblage and autotrophic flagellate and dinoflagellate dominated SCM on August 13, coinciding with greater vertical mixing of diatoms.

Estimated Grazer Abundance

Macro-zooplankton grazer densities were estimated only at A1. On the basis of Clarke-Bumpus tows, the daylight near surface herbivorous zooplankton component consisted primarily of the copepod *Acartia* spp. The abundance of this herbivore decline slightly between August 9 and 11, but then increased significantly between August 11 and 13. Greater depth dependence in the counts on August 13 also indicated this increase was accompanied by a less uniform vertical distribution that was centered near 5 m (Table 3).

Dynamics of Vertical Biomass Distribution

While the M2 time series was not detailed or long enough to observe any significant evolution in vertical biomass distribution, the synoptic picture of chlorophyll and NO_3^- distribution on May 17 does put some constraints on possible underlying dynamics for this period.

There is a factor of nine deficit in 0–4 m integrated Chl*a* relative to corresponding NO_3^- disappearance, compared with a relative mass deficit of only three at the SCM. This is consistent with a greater relative phytoplankton biomass export from the 0–4 m layer. Similarly, in situ growth as a sole source term for the May 17 SCM would require a net specific local growth rate of $2.7 \cdot d^{-1}$ (i.e. increase in 6 m [Chl*a*] from 1.4 to 20.2 mg Chl*a*·m^{-2} over 24 h). This is significantly higher than the $0.9 \cdot d^{-1}$ calculated from the chlorophyll normalized midday ^{14}C uptake rate of 1.5 mg C·mg(Chl*a*·h)$^{-1}$ (Fig. 4); assuming no sink terms, a C/Chl*a* of 20, and a 12-h day. Thus both limits to realistic growth rate and the depth dependence of the Chl*a* and NO_3^- mass divergence argues for an important nonlocal source term for the May 17 SCM. Two possible contributing terms are depth dependent sedimentation and a behavioral aggregation response. A depth (i.e. nutrient) dependent sinking rate would be inconsistent with the observed high NO_3^- levels, though in the absence of information about other nutrients the possibility remains (Bienfang et al. 1982). Similarly, evidence of active swimming avoidance of surface light levels has been demonstrated in strong swimming phytoplankters during times of nutrient stress (Blasco 1978; Heaney and Eppley 1981; Cullen et al. 1983). Though certainly circumstantial, two motile species (an unidentified cryptomonad and less importantly *M. rubrum*) did contribute, albeit in a relatively minor way, to phytoplankton biomass during May. Interestingly, *M. rubrum* is known to be a strong vertical migrator (Packard et al. 1978).

Given the 1–5 m current shear, it can also be argued that shear advection and related horizontal exchange processes contributed significantly to the Chl*a* and NO_3^- mass balance. Judging by the noneroding mixed layer after May 16, the strong mixing event observed at M2 was episodic and likely related to the few hours of high wind speed and current shear observed on May 16. Since the current shear was primarily wind driven and by inference restricted to above drogue depth, it appears that contributions by lateral exchange were probably limited to the upper 5 m (i.e. vertical mixing to 5 m driven by shear currents above drogue depth). Hence horizontal Chl*a* and NO_3^- imports and exports from advection probably did not contribute significantly to the 6 m deep SCM.

Prior to the SCM becoming depth constant at A1, the spatial relationship between the peaks appeared to be time dependent, with the biomass peak descending to ≈3 m after about 3 d, while the total production maximum (TPM) descended relatively slowly, eventually coinciding with the SCM only after the fifth day. Though the descent of the TPM was not obviously accompanied by a deepening nitracline, the time series was marked by an apparent surface mixing event and shoaling of the nitracline between August 11 and 13, which could have obscured a spatial relationship. Assuming for the present that the latter is the case, then the A1 SCM conforms in some ways to the deep chlorophyll maxima found at the base of deep oligotrophic mixed layers in the tropical ocean. It is usually located at or slightly below the TPM, which is itself associated with the nutricline (see Cullen 1982 for a review). If, as the evidence indicates, the SCM reached a steady state depth prior to the TPM, then some factor (or combination of factors) other than a direct and constant dependence on growth distribution was important in determining the steady state SCM depth.

Chl*a* and NO_3^- Mass Balance

In view of the plethora of competing processes that must have contributed in varying degrees to the observed evolution of Chl*a* and NO_3^- distribution at A1, it is useful to calculate Chl*a* and nitrogen budgets as a means of scaling the different terms. Since these component estimates are clearly dependent on each other,

TABLE 2. Dominant autotrophic species during the May and August studies. Cell volumes estimated by eyepiece micrometer averaged over all samples. Photo. fraction is the fractional contribution to the total autotrophic cell volume.

	Species	No./cm^3	Cell volume (μm^3)	Total volume (μm^3)	Photo. fraction
August 9	Depth = 0 m				
	Thalassiosira polychorda	19	$2.2\cdot10^4$	$4.2\cdot10^5$	0.29
	Gymnodinium sp.	35	$6.6\cdot10^3$	$2.3\cdot10^5$	0.14
	chrysophyte	226	$7.3\cdot10^2$	$1.6\cdot10^5$	0.10
	Mesodinium rubrum	8	$2.3\cdot10^4$	$1.8\cdot10^5$	0.11
	cryptomonad	320	$7.0\cdot10^2$	$2.2\cdot10^5$	0.13
	Peridinium sp.	8	$3.9\cdot10^4$	$3.1\cdot10^5$	0.19
	Chaetoceros spp.	19	$1.4\cdot10^3$	$2.7\cdot10^4$	0.02
August 9	Depth = 10 m				
	chrysophyte	394	$7.3\cdot10^2$	$2.9\cdot10^5$	0.38
	cryptomonad	250	$7.0\cdot10^2$	$1.8\cdot10^5$	0.24
	Gymnodinium sp.	16	$6.6\cdot10^3$	$1.1\cdot10^5$	0.14
August 11	Depth = 0 m				
	Peridinium sp.	6	$3.9\cdot10^4$	$2.3\cdot10^5$	0.18
	cryptomonad	219	$7.0\cdot10^2$	$1.5\cdot10^5$	0.12
	chrysophyte	169	$7.3\cdot10^2$	$1.2\cdot10^5$	0.10
	T. polychorda	3	$2.2\cdot10^4$	$6.6\cdot10^4$	0.05
	M. rubrum	14	$2.3\cdot10^4$	$3.2\cdot10^5$	0.25
	Gymnodinium sp.	33	$6.6\cdot10^3$	$2.2\cdot10^5$	0.17
	Chaetoceros spp.	4	$1.4\cdot10^3$	$6.4\cdot10^3$	0.00
August 11	Depth = 2.5				
	chrysophyte	1316	$7.3\cdot10^2$	$9.6\cdot10^5$	0.47
	Chaetoceros spp.	354	$1.4\cdot10^3$	$5.0\cdot10^5$	0.24
	cryptomonad	97	$7.0\cdot10^2$	$6.8\cdot10^4$	0.03
	M. rubrum	9	$2.3\cdot10^4$	$2.1\cdot10^5$	0.10
August 13	Depth = 0 m				
	chrysophyte	568	$7.3\cdot10^2$	$4.1\cdot10^5$	0.33
	Chaetoceros spp.	163	$1.3\cdot10^3$	$2.3\cdot10^5$	0.19
	M. rubrum	6	$2.3\cdot10^4$	$1.4\cdot10^5$	0.11
	cryptomonad	172	$7.0\cdot10^2$	$1.2\cdot10^5$	0.10
	Dinophysis sp.	1	$1.2\cdot10^5$	$1.2\cdot10^5$	0.10
	Peridinium sp.	3	$3.9\cdot10^4$	$1.2\cdot10^5$	0.10
August 13	Depth = 2.5 m				
	chrysophyte	1124	$7.3\cdot10^2$	$8.2\cdot10^5$	0.49
	M. rubrum	12	$2.3\cdot10^4$	$2.8\cdot10^5$	0.17
	T. polychorda	4	$2.2\cdot10^4$	$8.8\cdot10^4$	0.05
	Chaetoceros spp.	71	$1.4\cdot10^3$	$9.9\cdot10^4$	0.06
	cryptomonad	206	$7.0\cdot10^2$	$1.4\cdot10^5$	0.08
	Peridinium sp.	4	$3.9\cdot10^4$	$1.6\cdot10^5$	0.10
August 13	Depth = 3 m				
	chrysophyte	3513	$7.3\cdot10^2$	$2.6\cdot10^6$	0.75
	M. rubrum	8	$2.3\cdot10^4$	$1.8\cdot10^5$	0.05
	Chaetoceros spp.	201	$1.4\cdot10^3$	$2.8\cdot10^5$	0.08
	cryptomonad	254	$7.0\cdot10^2$	$1.8\cdot10^5$	0.05
	Peridinium sp.	5	$3.9\cdot10^4$	$2.0\cdot10^5$	0.06

an effort was made to organize the calculation such that empirically based terms or those for which a consensus has emerged were calculated first. Terms that are poorly constrained, either because this consensus is lacking or because they are geographically dependent, were calculated last.

Some independent verification of the calculation does accrue from the simultaneous balancing of the two separate budgets. The degree to which they converge is a qualitative measure of the accuracy of the calculation.

Nutrient replete C/N and C/Chl*a* of seven and 20 were assumed, respectively (Laws and Bannister 1980). Uptake rate from the in situ incubations was converted to daily net production by extrapolating to total daily PAR and assuming a nighttime (i.e. 8 h) phytoplankton respiratory loss equal to 25% of maximum photosynthetic rate (P^M). This loss rate is midway between the 10–70% range measured for a number of motile and nonmotile phytoplankton (Falkowski and Owens 1978). A P^M of 6 mg C(milligrams of Chl*a* per hour) was obtained by fitting the *P–I* model equation of Platt and Gallegos (1981) to the A1 0–2 m (i.e. surface mixed layer) in situ carbon uptake rates for cases where $[NO_3^-] > 1$ μM. The underlying assumption was that these 0–2 m populations were light limited and uniformly mixed. Since carbon uptake was measured every other day at A1, it was necessary to linearly interpolate between these days in calculating accumulated net growth. Also, the effect of the uncertainty in August 13 SCM biomass, due to the discrepancy between extracted Chl*a* and in vivo fluorescence, was tested by calibrating the fluorescence signal using the

TABLE 3. Daytime herbivorous copepod distribution at drift station A1. Grazing and nitrogen excretion (expressed as NO_3^-) based on a clearance rate of 3 $mL \cdot d^{-1}$ and 60% N recycling.

Date	Depth (m)	No./m^3	Grazing ($\mu g\ Chl a \cdot m^{-3} \cdot d^{-1}$)	N-excretion ($\mu g\ NO_3^- \cdot m^{-3} \cdot d^{-1}$)
August 9	2	458	7.4	12.7
	8	104	0.7	1.2
	10	238	1.5	2.6
	16	158	0.1	0.2
	20	37	< 0.1	—
0–6 m mean			6.3	10.8
August 11	1	189	3.4	5.8
	3	192	6.9	11.8
	5	253	4.8	8.2
	20	203	0.3	0.5
0–6 m mean			5.0	8.6
August 13	1	120	1.7	2.9
	2	243	3.3	5.7
	3	77	1.9	3.3
	4	313	43.1*	73.7
	5	3794	43.1*	73.7
	8	1202	2.9	5.0
	20	243	< 0.1	—
0–6 m mean			19.3	32.9

*Based on mean of 4 and 5 m grazer density and [Chl*a*].

August 11 fluorescence yield to allow a second estimate of the August 13 SCM [Chl*a*]. Equivalent daily grazing loss and NO_3^- recycling was calculated on the basis of observed (daytime) copepod densities, a representative constant clearance rate of 3.0 $mL \cdot d^{-1}$ and a 60% N-excretion ratio (e.g. Raymont 1983). Since NO_3^- is the only ambient nitrogen pool in this initial mass balance calculation, organic nitrogen was assumed to remineralized in the form of NO_3^-. The calculated average 0–6 m grazing and recycled nitrogen fluxes are presented in Table 3.

Summing the foregoing terms allows a mass balance calculation based only on fluxes that are at least partly constrained by directly measured quantities at A1. While a large defect is evident for both budgets, it is much larger in the case of Chl*a* (Table 4). Net growth was predicted to be 161 mg $Chl a \cdot m^{-2}$ over 5 d. Biomass, on the other hand, either remained unchanged or increased by 15 mg $Chl a \cdot m^{-2}$ over the 5 d depending on the choice of August 13 SCM biomass. By comparison, NO_3^- agreed to within a factor of three, with a predicted consumption of 459 mg $NO_3^- \cdot m^{-2}$ compared with the measured decline of 150 mg $NO_3^- \cdot m^{-2}$. A further relative reduction in the NO_3^- defect can be effected by correcting for the apparent upwelling at A1 on August 13. Based on changes in temperature distribution, the uplifting of the water column between August 11 and 13 appears to have been ≈0.5 m (Fig. 3). The vertical NO_3^- transport, based on this shoaling and the difference in mean 0–3.5 and 3.5–6 m [NO_3^-] (i.e. there were no 4-m measurements), was ≈23 mg $NO_3^- \cdot m^{-2}$. The same calculation can account for only ≈2 mg $Chl a \cdot m^{-2}$ of missing biomass, due to differences in gradient across the pycnocline. Thus, assuming a N/Chl*a* of 20/7, the observed Chl*a* increase is a factor of three to four less than the observed NO_3^- decrease, and the corresponding mass balance defects (i.e. prediction–observation) differ by a factor between one and two.

The mass balance defects can be ameliorated by invoking additional process terms not supported by direct evidence. In the foregoing calculation, no distinction was made between moities in the ambient nitrogen pool. Hence, in order for calculated nitrogen demand and NO_3^- disappearance to be comparable quantities, nitrogen (i.e. in this case NO_3^-) recycling needed to be treated explicitly. An alternative is to convert the total primary production being measured by carbon uptake to new (i.e. NO_3^- based) production. In this case nitrogen recycling is no longer an explicit term in the NO_3^- mass balance, while still retaining the simplicity of a single ambient nitrogen moity. However, new uncertainty arises concerning the conversion from total to new production. No direct empirical conversion is possible. Thus an approximation must be made on the basis of a general empirical relationship between the ratio of new to total production and ambient [NO_3^-], established by Harrison et al. (1987) for coastal waters. Though variation was evident between regions, due to among other things the ambient [NH_4^+], generally speaking for [NO_3^-] >0.4 μM, regenerated production accounts for 40% of the total. Correcting by this factor decreases the estimated NO_3^- demand at A1 to 276 mg $NO_3^- \cdot m^{-2}$, thereby reducing the NO_3^- mass defect to less than a factor of two.

In the absence of measured phytoplankton sinking rate, an estimate of the phytoplankton sedimentation flux can be approximated on the basis of cell size. The dominant phytoplankters at A1 ranged in size from ≈$8 \cdot 10^3$ μm^3 to ≈$7 \cdot 10^4$ μm^3 corresponding to size-dependent sinking rates of 0.3 to 1 $m \cdot d^{-1}$ under nutrient replete conditions (Bienfang and Harrison 1984). Using 1 $m \cdot d^{-1}$ and the highest observed 0–4 m [Chl*a*], total sedimentation at A1 was 44.4 mg $Chl a \cdot m^{-2}$ or ≈30% of the missing biomass. This estimate represents an upper limit to the degree to which A1 can be characterized as nutrient replete.

Though micro-heterotrophic grazing is not likely to be as important as in oceanic regions (Beers and Stewart 1967), the significant presence of naked flagellates in the phytoplankton makes this class of herbivore potentially important in the basin. In the absence of direct measurement, micro-heterotrophic grazing can be inferred indirectly from the suspended phaeopigment concentration (Welschmeyer and Lorenzen 1985). In

TABLE 4. The 0–4 m Chl*a* and NO_3^- mass balance for drift station A1, with nitrogen demand and phytoplankton growth calculated from phytoplankton carbon fixation rates after losses and gains due to daytime grazing, respectively. δNO_3^- and δChl*a* are the measured NO_3^- disappearance and Chl*a* increase at A1.

Date	*N*-demand [mg $N \cdot m^{-2}$]	δNO_3^- [$mg \cdot m^{-2}$]	Growth [mg Chl*a*·m^{-2}]	δChl*a* [$mg \cdot m^{-2}$]
August 9	70	—	24	—
10	85	—	30	—
11	99	361	35	19
12	139	—	49	—
13	66[a]	−211	23	−16(−4)[b]
Total	459	150	161	3(15)

[a] Production prior to sampling time.

[b] Based on change in SCM in vivo fluorescence.

TABLE 5. Calculated sink and source terms which reconcile the divergence between the drift station A1 Chl*a* and NO_3^- mass balances. Mass defect is the difference between the observed change in mass and that predicted from measured phytoplankton growth rate. Specific nitrogen remineralization terms (e.g. macrozooplankton daytime grazing) are assumed to enter a reduced nitrogen pool and hence not contribute to the NO_3^- mass balance calculation.

Budget term	NO_3^- [mg $NO_3^- \cdot m^{-2}$]	Chl*a* [mg Chl*a*·m^{-2}]
Mass defects from Table 4	309	158(146)*
Macrozooplankton daytime grazing	—	0.3
Microheterotroph grazing	—	8
Sedimentation	—	44
40% N recycling	184	—
Advection	23	2
Outstanding defect	102	103(92)

*Based on in vivo fluorescence August 13 biomass.

most cases phaeopigments were measured to be $<1\ mg \cdot m^{-3}$. The single exception was 5.1 $mg \cdot m^{-3}$ for the August 11 SCM, which converts to an equivalent of 8 mg Chl*a*·m^{-2}, (i.e. ≈5% of the deficit) using the conversion factor 0.66 (Shuman and Lorenzen 1975). This is a lower limit in as much as phaeopigment photo-destruction between August 9 and 11 was not accounted for.

The foregoing adjustments reduce the Chl*a* deficit to between 92 and 103 mg Chl*a*·m^{-2} (i.e. range due to conflicting estimates of the August 13 SCM) and the NO_3^- excess to 102 mg $NO_3^- \cdot m^{-2}$ (Table 5). Thus, with N/Chl*a* = 20/7, the adjusted Chl*a* deficit is now a factor of two to three greater than the NO_3^- excess. Most of this increase in between budget divergence arises from the lower NO_3^- demand after the conversion of total production to new production and hence is constrained by its degree of uncertainty.

The remaining mass balance defects, combined with the Chl*a* and NO_3^- pycnocline gradients (Table 6), can be used to estimate mixing across the pycnocline. An approximate maximum time averaged vertical diffusivity of $k_z \sim 10^{-4} \cdot m^{-2} \cdot s^{-1}$ has been calculated from upper water column salt extraction during buoyant tidal flow in the basin (Stucchi 1985). However, while the resulting Chl*a* mixing flux can account for 89 mg Chl*a* or 80–90% of the remaining mass deficit, a factor of 50 lower k_z (i.e. $4 \cdot 10^{-6}$) is consistent with the remaining NO_3^- excess. This difference still remains at least a factor of 10 if total primary production is used instead of new production. Hence uncertainty concerning the latter does not seriously qualify this contrast.

TABLE 6. NO_3^- and Chl*a* pycnocline gradients and Brunt-Väisällä frequency *N* for drift station A1.

Date	dChl*a*/dz [$mg \cdot m^{-4}$]	dNO_3^-/dz [$mg \cdot m^{-4}$]	*N* [$10^{-3} \cdot s^{-1}$]
August 9	0.4	68	10.0
10*	2.3	84	10.0
11	4.2	99	10.0
12*	2.9	59	11.8
13	1.6	19	13.5

*Interpolated estimates.

The lower k_z estimate can account for only ≈3% of the missing Chl*a*. However, it is within a factor of two of a theoretical relationship between internal wave dissipation and vertical diffusion, calibrated by data from a sill-fjord (i.e. $k_z \sim 1.2 \cdot 10^{-8} N^{-1.2}$, Svensson 1979). This relationship and the pycnocline brunt frequency *N* at A1 (Table 6) predicts $k_z \sim 3 \cdot 10^{-6} m^{-2} \cdot s^{-1}$).

Choosing between the two diffusion parameter estimates, and hence by inference choosing between diapycnal mixing due to tidal inflow versus (wind generated) internal waves, appears to rest on the relative likelihood of an additional NO_3^- as opposed to Chl*a* sink to compensate for resulting excess mass. The only plausible NO_3^- sink not considered to this point is horizontal exchange such as advection associated with the shallow current shear. It was suggested earlier that shear advection associated with the May 16 wind storm did not contribute significantly below the 5-m mixed layer. A wind storm also occurred on

August 13. The mixed layer associated with this weaker storm (i.e. 5 $m \cdot s^{-1}$ for a similar duration) was only 2 m instead 5 m, thereby similarly remaining above the SCM depth. If the current shear at A1 was positioned above drogue depth as in May, then advection would not have contributed to the SCM mass balance. Advection could have contributed at shallower depths, however, the 0–2 m [NO_3^-] was observed to increase on August 13, consistent with lateral import rather than export in the 2-m mixed layer (Fig. 6). This increase in [NO_3^-] accounts for ≈70% of the excess NO_3^- (Table 4), and hence the NO_3^- budget would be nearly balanced without the need for a vertical transport term. While this possibility cannot be discounted, in terms of choosing between the two vertical mixing regimes it would argue even more strongly for the lower k_z estimate.

The alternative to an additional NO_3^- sink is an additional Chl*a* sink. Nighttime changes in the zooplankton community were not measured at A1. However, qualitative nighttime ≈3-m depth net tows in Holberg Inlet during September 1982 (ICM 1983) indicated a community dominated on a wet weight basis by *C. pacificus*, *M. pacifica*, and *Euphausia pacifica*, an assemblage quite different from the daytime *Acartia* spp. dominated one at A1. These grazers have clearance rates three to >30 higher than *Acartia* (Peters and Downing 1984) and could, in moderate densities, provide grazing pressure that is the necessary factor of 10^3 greater than that based on daytime herbivore abundance.

Alternatively, if the assumption of sufficient ambient nutrient levels is dropped, then the sedimentation velocity used in the earlier calculation may be an underestimate. Though particular details about the underlying experimental protocol may be important (e.g. Bienfang et al. 1982), sinking rates of senescent *Chaetoceros* spp., for example, have been reported that are a factor of three greater than the one used here (Smayda 1970). Cell senescence is inconsistent with the ambient [NO_3^-] during A1. However, a sinking rate of this order would be sufficient to balance the Chl*a* budget. Furthermore, extrapolating the calculated ≈150 mg $Chla \cdot m^{-2}$ over a March–October growing season results in an annual bottom flux (ignoring water column losses) of ≈140 gm $C \cdot m^{-2} \cdot yr^{-1}$ again assuming a C/Chl*a* of 20. This quasi-annual deposition is in line with annual estimates for other coastal embayments such as Departure Bay, Vancouver Island (200 gm $C \cdot m^{-2} \cdot yr^{-1}$, Stephens et al. 1967), Bedford Basin, Nova Scotia (91 gm $C \cdot m^{-2} \cdot yr^{-1}$, Hargrave et al. 1976) and St. Margaret's Bay (134 gm $C \cdot m^{-2} \cdot yr^{-1}$, Webster et al. 1975).

Discussion

On the assumption that the May and August studies were not seriously compromised by horizontal exchange influences, the time dependent vertical phytoplankton distribution, its apparent relationship to mixing conditions, and the estimated relative magnitudes of the most probable Chl*a* and NO_3^- flux terms, present a relatively coherent, albeit incomplete, picture of coupling between basin mixing and phytoplankton standing stock.

The mass budgets indicate that nocturnal grazing is probably the single most important biomass sink, while diapycnal mixing is the most important NO_3^- source. More significantly, the calculation implicates a vertical mixing regime in the surface layer at A1 that was one to two orders of magnitude lower than would be expected from diffusive tidal mixing, but that agreed to within a factor of two with an internal wave dissipation estimate. This conclusion is not qualified by the relative importance of lateral NO_3^- input. Invoking this term would require a further reduction in k_z, thus bringing it further in agreement with the lower k_z prediction. The resulting low mass balancing mixing rate, combined with the apparent dominant role by wind stress in the observed surface mixing and the apparent lack of significant erosion of the pycnocline during periods of seasonally extreme wind stress, suggests that seasonally dependent tidal mixing in the surface layer contributes to the seasonal biomass–stability relationship in the historical data set.

Established theory concerning the control of relative tidal inflow buoyancy in the basin implicitly states that, from fall to the following spring, the lack of coastal upwelling and the widespread distribution of surface freshwater input along the Vancouver Island coast is such, that tidal inflow becomes positively buoyant in spite of a concomitant density decrease in basin surface water (Stucchi and Farmer 1976). Accordingly, it would be reasonable to expect increased basin water stability (i.e. that resulting from increased surface freshwater flux) to be associated with an increased predominance by a buoyant tidal inflow mixing regime, or more specifically an increased tidal contribution to surface mixing. In summer, by contrast, the seasonal appearance of coastal upwelling results in tidal inflow that is usually negatively buoyant regardless of the surface buoyancy flux. Under these conditions, an increase in the surface buoyancy input tends to increase, rather than decrease, the relative density of the inflow, causing it to descend into deepwater closer to the basin entrance and hence interact less with basin surface water. As a consequence, increased static stability of the basin surface layer in summer will tend to be associated with a decrease in tidal contribution to surface mixing, a decrease in vertical mixing, and an increase in pycnocline accumulation of phytoplankton.

A SCM located well above compensation depth appears to be a regular occurrence in the basin. This apparently long-lived feature does not appear to conform with the light limited steady state balance between net growth, sinking, and diffusion type SCM, first discussed theoretically by Riley et al. (1949) and later elaborated on by Parslow (1981), nor does it appear to constitute the nutrient trap type of SCM (Jamart et al. 1977; Parslow 1981). The important contribution by motile phytoplankton to SCM biomass in both May and August suggests an aggregation response mechanism for the SCM (Cullen 1982). This is suggested further by the apparent increase in the predominance of motile phytoplankton in the SCM after a wind mixing event in August. In the absence of any obvious nutrient depletion, nutrition alone does not appear to be a plausible explanation for migratory behavior. However, vertical migration has been observed in response to both nutrient stress and high light levels (e.g. Blasco 1978). An avoidance of surface light levels due to some nutritional limitation would also be consistent with nutritionally based elevated sedimentation flux balancing of the Chl*a* mass balance.

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References

BEERS, J. R., AND G. L. STEWART. 1967. Micro-zooplankton in the euphotic zone at five locations across the California current. J. Fish. Res. Board Can. 24: 2053–2067.

BIENFANG, P. K., AND P. J. HARRISON. 1984. Co-variation of sinking rate and cell quota among nutrient replete marine phytoplankton. Mar. Ecol. Prog. Ser. 14: 297–300.

BIENFANG, P. K., P. J. HARRISON, AND L. M. QUARMBY. 1982. Sinking rate response to depletion of nitrate, phosphate, and silicate in four marine diatoms. Mar. Biol. 67: 295–302.

BLASCO, D. 1978. Observations on the diel migration of marine dinoflagellates off the Baja California coast. Mar. Biol. 46: 41–47.

BUCKLEY, J. R. 1977. The currents, winds, and tides of northern Howe Sound. PhD. thesis, University of British Columbia, 226 p.

CULLEN, J. J. 1982. The deep chlorophyll maximum: comparing vertical profiles of chlorophyll *a*. Can. J. Fish. Aquat. Sci. 39: 791–803.

CULLEN, J. J., E. STEWART, E. RENGER, R. W. EPPLEY, AND C. D. WINANT. 1983. Vertical motion of the thermocline, nitracline and chlorophyll maximum layers in relation to currents on the Southern California Shelf. J. Mar. Res. 41: 239–262.

FALKOWSKI, P. G., AND T. G. OWENS. 1978. Effects of light intensity in photosynthesis and dark respiration in six species of marine phytoplankton. Mar. Biol. 45: 239–295.

HARGRAVE, B. T., G. A. PHILLIPS, AND S. TAGUCHI. 1976. Sedimentation measurements in Bedford Basin, 1973–74. Fish. Mar. Ser. Rep. 608 129 p.

HARRISON, W. G., T. PLATT, AND M. R. LEWIS. 1987. *f*-Ratio and its relationship to ambient nitrate concentration in coastal waters. J. Plank. Res. 9: 235–248.

HEANEY, S. L., AND R. W. EPPLEY. 1981. Light, temperature and nitrogen as interacting factors affecting diel vertical migrations of dinoflagellates in culture. J. Plank. Res. 3: 331–344.

ICM (ISLAND COPPER MINE). 1983. Island Copper Mine 1982 Annual Environmental Assessment Rep. Island Copper Mine, Port Hardy, B.C. 106 p.

JAMART, B. M., D. F. WINTER, K. BANSE, G. C. ANDERSON, AND R. K. LAM. 1977. A theoretical study of phytoplankton growth and nutrient distribution in the Pacific Ocean off the northwestern U.S. coast. Deep Sea Res. 24: 753–773.

JEFFERY, S. W., AND G. F. HUMPHREY. 1975. New spectrophotometric equations for determining chlorophylls a,b,c_1, and c_2 in higher plants, algae, and natural phytoplankton. Biochem. Physiol. Pflanz. 167: 191–194.

KESSLER, T. A., AND T. R. PARSONS. 1989. Primary production in a tidally energetic fjord: evidence of seasonal and inter-annual tidal forcing. Can. J. Fish. Aquat. Sci. 46: (This issue)

LAWS, E. A., AND T. T. BANNISTER. 1980. Nutrient and light limited growth of *Thalassiosira fluviatilis* in continuous culture with implications for phytoplankton growth in the ocean. Limnol. Oceanogr. 25: 457–473.

LEWIS, M. R., AND T. PLATT. 1982. Scales of variability in estuarine ecosystems, p. 3–20. *In* Estuarine comparisons. V. S. Kennedy [ed.] Proceedings of the 6th Biennial Int. Est. Res. Con. Gleneden Beach Oregon, Nov. 1981. Academic Press Paris.

PACKARD, T. T., D. BLASCO, AND R. T. BARBER. 1978. *M. rubrum* in the Baja California upwelling system, p. 73–89. *In* Upwelling ecosystems, R. Boje and M. Tomczuk [ed.] Springer-Verlag Berlin.

PARSLOW, J. S. 1981. Phytoplankton–zooplankton interactions: data analysis and modelling (with particular reference to Ocean Station P (50°N, 145°W) and controlled ecosystem experiments). PhD. thesis, University of British Columbia, 400 p.

PETERS, R. H., AND J. A. DOWNING. 1984. Empirical analysis of zooplankton filtering and feeding rates. Limnol. Oceanogr. 29: 763–784.

PLATT, T., AND C. L. GALLEGOS. 1981. Photosynthesis measurements on natural populations of phytoplankton: numerical analysis. *In* Physiological bases of phytoplankton ecology. T. Platt [ed.] Can. Bull. Fish. Aquat. Sci. 210 346 p.

RAYMONT, J. E. G. 1983. Plankton and productivity in the oceans, 2nd ed. Vol. 2 Zooplankton. Pergamon Press Oxford, 824 p.

RILEY, G. A., H. STOMMEL, AND D. F. BUMPUS. 1949. Quantitative ecology of the plankton of the Western North Atlantic. Bull. Bing. Oceanogr. Coll. 12: 1–169.

SHUMAN, F. R., AND C. J. LORENZEN. 1975. Quantitative degradation of chlorophyll by a marine herbivore. Limnol. Oceanogr. 20: 580–586.

SMAYDA, T. J. 1970. The suspension and sinking of phytoplankton in the sea. Oceanogr. Mar. Biol. Ann. Rev. 8: 353–414.

STEPHENS, K., R. H. SHELDON, AND T. R. PARSONS. 1967. Seasonal variations in the availability of food for benthos in a coastal environment. Ecology 48: 852–855.

STRICKLAND, J. D. H., AND T. R. PARSONS. 1972. A practical handbook of seawater analysis. J. Fish. Res. Board Can. Bull. 167. 311 p.

STUCCHI, D. 1985. The tidal jet in Rupert-Holberg Inlets, B.C. Atmos. Ocean. 23: 118–136.

STUCCHI, D., AND D. M. FARMER. 1976. Deep-water exchange in Rupert-Holberg Inlets. Inst. Ocean. Sci., Patricia Bay Pac. Mar. Sci. Rep. 76-10.

SVENSSON, T. 1979. Tracer measurements of mixing in the deep water of a small stratified sill fjord, p. 233–240. *In* Fjord oceanography. H. J. Freeland, D. M. Farmer, and C. D. Levings [ed.] Plenum Press, New York, NY.

WEBSTER, T. J. M., M. PARANJAPE, AND K. H. MANN. 1975. Sedimentation of organic matter in St. Margaret's Bay, Nova Scotia. J. Fish. Res. Board Can. 32: 1399–1407.

WELSCHMEYER, N. A., AND C. J. LORENZEN. 1985. Chlorophyll budgets: zooplankton grazing and phytoplankton growth in a temperate fjord and the Central Pacific Gyre. Limnol. Oceanogr. 30: 1–21.

Water Column Methylmercury in the Wabigoon/English River–Lake System: Factors Controlling Concentrations, Speciation, and Net Production[1]

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Ten years after major releases of inorganic mercury from a chloralkalai plant were sharply reduced, methylmercury (MeHg) levels remain markedly elevated in waters in most of the Wabigoon River system downstream of Dryden, Ontario, Canada. The water column appears to be a very important site for methylation and demethylation. In water, MeHg and inorganic Hg appear to be in quasi equilibrium, as the MeHg : total mercury ratio is independent of contact time with sediments, the atmosphere, and the theoretical residence time of waters. The MeHg : total Hg ratio increased with distance downstream of Dryden, but MeHg always averaged less than 10% of the total Hg concentration. MeHg was mostly soluble, as defined by field centrifugation. Net production of MeHg appeared to be directly related to the amount of MeHg required to establish and maintain equilibrium conditions with inorganic Hg. A model is developed which adequately describes water column MeHg concentrations. Considerations of the model suggests different restoration strategies for different parts of the Wabigoon/English River–Lake system.

Dix ans après que les importantes quantités de mercure inorganique déversées à partir d'une fabrique de chlore et de soude caustique ont diminué considérablement, les concentrations de méthylmercure (MeHg) demeurent néanmoins très élevées dans presque toute la rivière Wabigoon en aval de Dryden, en Ontario, au Canada. La colonne d'eau paraît être un important lieu de méthylation et de déméthylation. Le méthyle et le mercure inorganique semblent être en quasi-équilibre, car le rapport MeHg-mercure total est indépendant de la durée du contact avec les sédiments et l'atmosphère et du temps de séjour théorique des eaux. Ce rapport augmentait en fonction de la distance en aval de Dryden, mais MeHg était toujours, en moyenne, inférieur à 10 pour cent de la concentration totale de mercure. Le MeHg était la plupart du temps soluble, comme l'ont révélé les centrifugations effectuées sur le terrain. La production nette de MeHg semblait directement liée à la quantité de MeHg nécessaire pour établir et maintenir l'état d'équilibre avec le mercure inorganique. On a élaboré un modèle qui décrit adéquatement les concentrations de méthylmercure dans la colonne d'eau. Les données de ce modèle semblent indiquer qu'il faudrait appliquer différentes stratégies de restauration selon la partie considérée du réseau des rivières Wabigoon et des Anglais.

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Although the biogeochemical cycle of mercury (Hg) has received substantial attention, critical gaps remain in our understanding of certain components of the Hg cycle. There is evidence that most of the Hg in fish is methylmercury (MeHg) and that MeHg levels in water largely govern Hg body burdens in top predatory fish (Parks and Hamilton 1987). However, there is a general lack of information on factors controlling MeHg concentrations, speciation, and net production in waters.

This paper attempts to reconcile observations from two studies on MeHg in waters of the Wabigoon River system, Ontario, Canada, from 1978 to 1980. The Wabigoon River system has been seriously contaminated by the discharge of approximately 10 tonnes of inorganic Hg from a chloralkalai plant at Dryden, Ontario, between 1962 and 1970 (Armstrong and Hamilton 1973; Jackson and Woychuk 1980; Allan et al. 1984). Although Hg discharges from the industrial complex were reduced by 99% in the early 1970's (Parks et al. 1984), biota in the system remain seriously contaminated, with sportfish such as walleye (*Stizostedion vitreum*) and northern pike (*Esox lucius*) frequently reported at 5–10 times background concentrations (Parks et al. 1984).

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[1] The views expressed are those of the authors and do not necessarily reflect the views of the Ontario Ministry of the Environment or other agencies. No endorsement should be inferred.

This study evaluates factors affecting water column concentrations of MeHg and suggests that water column concentrations in the Wabigoon system can be modeled within a sediment-water – suspended solids partition coefficient protocol.

Study Area

The Wabigoon/English River–Lake system is located in an area of substantial clay deposits on the Canadian Shield of northwestern Ontario (Fig. 1). Surficial sediments in contaminated regions of the Wabigoon River system have Hg concentrations which are typically 100–1000 times higher than the usual background concentrations of 0.01–0.05 μg/g dry weight (Armstrong and Hamilton 1973; Parks 1976; Jackson and Woychuk 1980; Parks et al. 1984).

Flows increased over an order of magnitude down the river system (Fig. 1), averaging 14 m^3/s at Dryden (1970–82), 46 m^3/s at Quibell (1953–82), 78 m^3/s at Ball Lake inflow (prorated), and 415 m^3/s at Ball Lake outflow (prorated) (Water Survey of Canada 1983; Parks et al. 1984). Excluding falls, the river gradient is less than 0.3 m/km. Time of travel measurements (using standard dye dilution techniques) for the Wabigoon River between Dryden and Clay Lake ranged from about 3 d in the spring runoffs of 1978 and 1979 to 27 d in the late summer of 1980 (Ontario Water Resources Commission 1970; Parks et al. 1984, 1986; P. Berg-Johannessen, Great Lakes Forest Products Ltd., unpubl. data). Average time-of-travel for the Dryden to Clay Lake reach is approximately 5 d (Parks et al. 1984).

Clay Lake encompasses 3000 ha and has a volume of $2.4 \times 10^8/m^3$ and a mean depth of 8 m. The theoretical residence time, calculated from prorated flows from the hydrological station at Quibell Falls, was 57 d at an annual mean flow of 49 m^3/s (1953–76) and was approximately 31 d using mean May flows. During drought conditions in 1980, the theoretical residence time for Clay Lake was 3 times longer in May and 10 times longer in June than the long-term values for these months. The western basin is dimictic whereas the eastern basin does not stratify.

Methods

Study Sites

Sampling sites were located between Dryden and the outflow of Clay Lake for the first study year (Fig. 1). A reference site (Site 1) is situated at the outflow of Wabigoon Lake, upstream from any known industrial discharge. Sites 3 and 4, at the inflow and outflow of Wainwright Reservoir, respectively, were established to determine the effect of the reservoir on MeHg mobility and form. Sites 12–15 represent the inlet, eastern and western basins, and outlet of Clay Lake. Contaminated Sites 6, 16, 17, and 18 and reference Site 2 were added in the second year, July 1979 – June 1980.

Sampling and Analytical Protocol

For the first year (August 1978 – June 1979), unfiltered water samples were obtained monthly at midstream with an acid-washed acrylic "Van Dorn" or "Kenmerer" sampler at Sites 1, 3, 4, 13, and 14. For Clay Lake (Site 14), composite samples mixing equal volumes of water from the top, middle, and bottom of each limnetic zone were collected. In order to estimate the mass of MeHg in Clay Lake the sampling program was modified in the fall of 1978 to obtain samples 1 m below the lake surface, 1 m above lake bottom, plus a volume-weighted composite sample from the entire water column. Unfiltered "spot" samples were obtained from the effluent at Great Lakes Forest Products Ltd. and from the Dryden sewage plant which utilizes secondary treatment. Samples were collected weekly at Sites 12 and 15 during the ice-free season and more frequently

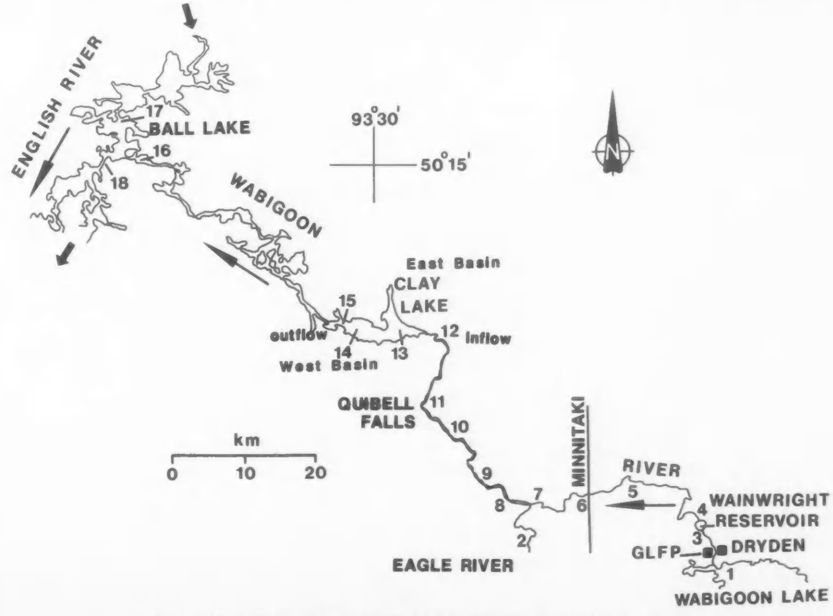

FIG. 1. Sampling sites in the Wabigoon/English River–Lake system.

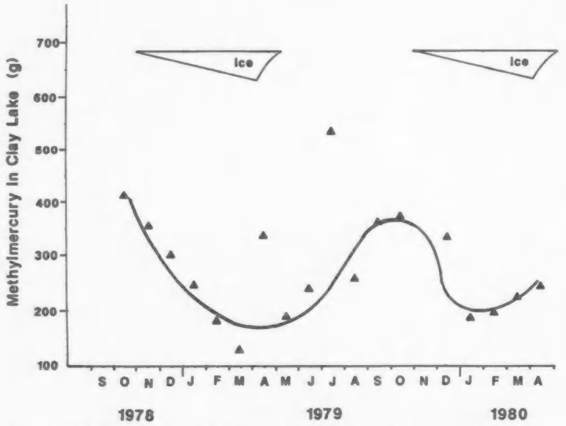

FIG. 2. MeHg content of Clay Lake waters, October 1978 – April 1980. Curve was fitted by eye. The lake content was calculated from lake volume and concentrations for the eastern basin (Site 12) in conjunction with results for a volume-weighted estimate for the entire western basin (Site 13).

at Site 12 during spring runoff. In the second year, July 1979 – June 1980, monthly samples were also taken at Sites 2, 6, 16, 17, and 18. During July–November 1980, weekly samples were obtained at Sites 1–15. Analyses for chemical parameters other than Hg followed the procedures of Parks et al. (1984).

MeHg and Total Hg Analyses

All analyses for MeHg and total Hg were conducted on unfiltered waters. Aliquots of samples intended for MeHg analysis were placed into 8-L polyethylene (Nalgene) bottles and preserved with 25 mL of sulphuric acid. The detection limit and precision is 0.2 ng/L for an 8-L sample (Furutani and Rudd 1980). Analytical procedures for total Hg followed Parks et al. (1986). ''Dissolved'' and ''particulate'' MeHg in water was defined by continuous-flow field centrifugation (Envirodata ''Sedisamp'') following Jackson et al. (1982).

MeHg Budgets

MeHg budgets were derived from MeHg concentrations and daily flow data supplied by the Water Survey of Canada. Site-specific discharge was prorated from the nearest station (Parks et al. 1986). When MeHg concentrations were below the detection limit of 0.2 ng/L, the value of 0.1 ng/L was used to estimate loadings. Net production of anthropogenically related MeHg was determined by subracting background MeHg loads. Net MeHg production is probably underestimated due to volatilization from contaminated sites. Mass balance was also used to evaluate seasonal differences in production. These were only significant for Clay Lake. Changes in whole-lake MeHg mass were obtained from Fig. 2. ''Variability'' of data for Clay Lake are presented in Table 1.

Results

Geographical Trends

MeHg concentrations in surface waters of the contaminated watercourse were higher than at reference sites (Table 2). Under normal hydrological conditions, the highest MeHg concentrations were observed near Clay Lake 80 km downstream of the location of most highly contaminated sediments (Parks et al. 1980; Jackson and Woychuk 1980; Jackson et al. 1982; Parks et al. 1984). Only 45–50% of the MeHg values at reference Sites, 1, 2, and 17 were above the detection limit of 0.2 ng/L. By assigning a value of 0.1 ng/L to samples below the detection limit, reference site concentrations average about 0.2–0.3 ng/L.

MeHg levels in effluent from Great Lakes Forest Products Ltd. in Dryden were usually low, ranging from <0.2 to 2.1 ng/L (Table 3). Low levels were also recorded in effluents from the Dryden municipal sewage treatment plant which utilizes conventional secondary treatment. Four grab samples collected in June and July 1979 yielded results averaging 0.47 ng/L (range 0.3–0.7 ng/L). MeHg loaded from the treatment plant was considered unimportant to river MeHg values because of the low volume from the sewage treatment plant (typically 0.050 m^3/s, N. Jarvis, Ontario Ministry of the Environment, pers. comm.). Low MeHg concentrations at the inflow to the Wainwright Dam (Site 3, Table 2) support the relative insignificance of industrial and municipal MeHg loadings. Concentrations at the outflow of the dam (Site 4) and at Minnitaki (Site 6) were similar to those at the dam inflow.

At the inflow to Clay Lake (Site 12) the mean MeHg concentration (1.4 ng/L) was nearly four times the average at Minnitaki (Site 6) (0.44 ng/L) (Table 2), suggesting that significant methylation had occurred between these sites. There was little change in mean MeHg concentrations in the rest of the Wabigoon River system as far as the inflow to Ball Lake, Site 16. Concentrations were usually near the detection limit at the outflow of Ball Lake (the confluence of the Wabigoon River and the uncontaminated English River).

For the 10 sampling periods encompassing the low-flow period of August–October 1980, mean MeHg concentrations at most contaminated sites averaged 2–4 ng/L (Fig. 3). Concentrations at the Wainwright Dam outflow (Site 4) averaged about three times those at the inflow. However, concentrations at the next site downstream (Site 5) were similar to those at the Clay Lake inflow.

Seasonal Trends

MeHg concentrations fluctuated by an order of magnitude at most sites (Fig. 4–6). Concentrations slightly above 0.2 ng/L often occurred at uncontaminated sites (Wabigoon Lake, Eagle River) during warm months such as August and September 1978 and May and June 1980. MeHg concentrations at the Wainwright Dam (Sites 3 and 4) did not show any seasonal trends. Trends are evident in Clay Lake. At the inflow and eastern basin of Clay Lake, highest values were recorded in summer and fall and lowest values in late winter and early spring. Concentrations at the inflow varied annually by an order of magnitude, with annual maxima of 3–4 ng/L.

MeHg concentrations in the western basin of Clay Lake peaked later in the fall than in the eastern basin (Fig. 5), probably because of internal cycling of Hg from the hypolimnion. MeHg concentrations at 1 m above the bottom of Clay Lake were often much higher than values at the surface, sometimes by an order of magnitude. Highest MeHg concentrations were observed in the hypolimnion in late summer when dissolved oxygen was zero (see Fig. 17). At lake turnover, MeHg levels were relatively uniform over depth. After fall turnover, concentrations increased during the winter. In both years, the win-

TABLE 1. Variably in MeHg concentrations of duplicate samples (unfiltered) collected during the first year of the study where 95% of the results were above the detection limit of 0.2 ng/L.

Site	Number of samples collected in duplicate	% of analyses above detection limit	Mean standard deviation of duplicate sample means (ng/L)	% C.V.
12. Wabigoon River, inflow to Clay Lake	37	96	0.056	4.0
13. Clay Lake, eastern basin	19	97	0.161	12.4
14. Clay Lake, western basin, epilimnion	18	97	0.229	19.0
15. Wabigoon River, outflow of Clay Lake	36	97	0.154	11.8

TABLE 2. Mean MeHg concentrations (ng/L) for monitoring sites in the Wabigoon/English River system, for two 12-mo periods, August 1978–June 1980. Sites 1, 2, and 17 are uncontaminated.

	August 1978 – July 1979			July 1979 – June 1980			
Site	*n*	Mean[a]	Range of monthly means	*n*	Mean[a]	Range of monthly means	Mean for two periods
1. Wabigoon Lake	25	0.22 (0.18–0.26)	0.1–0.35	16	0.32 (0.26–0.38)	0.1–0.5	0.27
2. Eagle River				23	0.18 (0.11–0.25)	0.1–0.6	
3. Wabigoon River, inflow to Wainwright Dam	25	0.61 (0.56–0.63)	0.1–1.8	13	0.24 (0.20–0.28)	0.1–0.9	0.43
4. Wabigoon River, outflow to Wainwright Dam	25	0.50 (0.46–0.54)	0.1–1.8	13	0.26 (0.21–0.31)	0.1–1.1	0.38
6. Wabigoon River at Minnitaki				14	0.44 (0.40–0.48)	0.1–1.7	
12. Wabigoon River, inflow to Clay Lake	61	1.3	0.6–2.2	27	1.5	0.3–7.4	1.4
13. Clay Lake, eastern basin	26	1.4	0.5–2.3	12	1.2	0.2–4.1	1.3
14. Clay Lake, western basin	25	1.3	0.4–2.2	11	1.2	0.3–2.1	1.2
15. Wabigoon River, outflow of Clay Lake	50	1.3	0.4–2.5	24	1.3	0.4–1.9	1.3
16. Wabigoon River, inflow to Ball Lake				12	1.0	0.4–2.1	
17. English River, to Ball Lake				12	0.25 (0.22–0.28)	0.1–0.5	
18. English River, outflow to Ball Lake				12	0.22 (0.17–.27)	0.1–0.4	

[a] >10% of the MeHg results were below the detection limit of 0.2 ng/L; therefore, upper and lower bounds on the means are given. The upper one equates the "less than" value to 0.2 ng/L and the lower one equates it to 0.

TABLE 3. MeHg concentrations (ng/L) in unfiltered effluent from Great Lakes Forst Products Ltd., Dryden.

	Grab samples	Composite samples[a]
Sept. 27, 1978	1.0	
Aug. 3, 1979	<0.2	
Oct. 3, 1979	2.1	
Dec. 19, 1979	0.3	<0.2
Jan. 9, 1980	<0.2	<0.2
Feb. 4, 1980	<0.2	<0.2
May 6, 1980		<0.2
June 4, 1980		0.5
June 11, 1980		0.4

[a] Represents a 24-h composite, with aliquots obtained every 20 min.

ter peak was 32% of the peak value observed during the preceding fall.

At the outlet of Clay Lake (Site 15), MeHg levels in the fall doubled during lake turnover. Levels at this site were also high in summer and fall and lower in winter and spring. Weekly fluctuations in concentrations were more moderate than those at the inflow to Clay Lake (Fig. 5).

Relationship between Temperature and MeHg

MeHg was significantly correlated with temperature for Wainwright Dam and Clay Lake (Sites 4, 12, 13, 14, and 15) (Table 4). At the inflow to Clay Lake, there was a strong positive relationship between MeHg and temperature over a wide range of flow conditions (Fig. 6). This positive association contrasts with the strong negative relationship between flow and MeHg concentration ($r = -0.48$, $n = 65$, $p < 0.001$) (Parks et al. 1984).

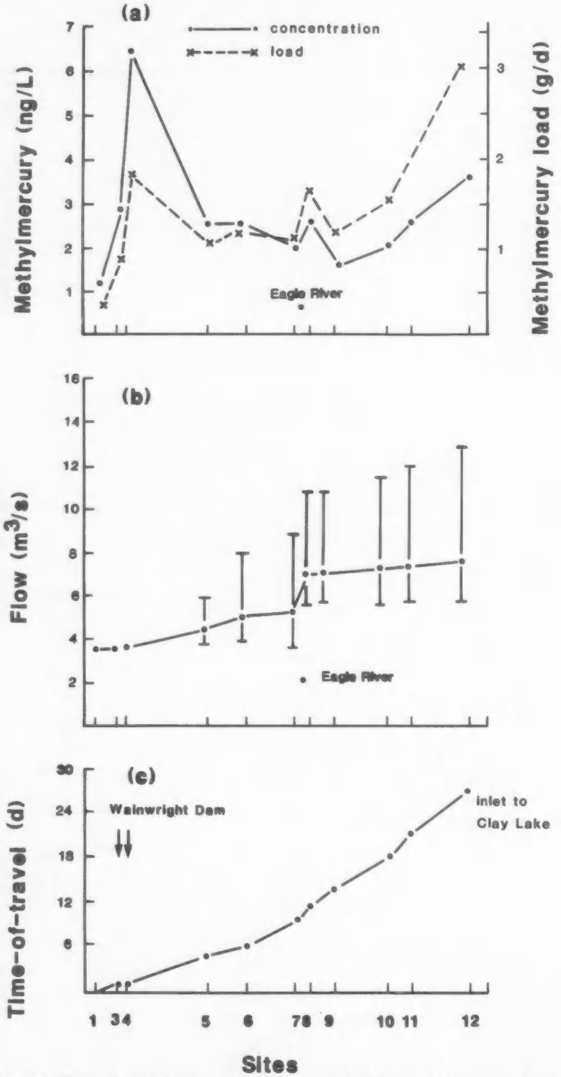

FIG. 3. Mean (a) MeHg concentrations and loadings, (b) flow, and (c) time-of-travel from Wabigoon Lake to Clay Lake inflow. Means of 10 samplings from August 4 to October 20, 1980, are shown. Vertical bars in Fig. 3b represent the range of flows.

Relationship between Dissolved Oxygen and MeHg

MeHg concentrations in water were higher under anoxic conditions versus aerobic conditions in the western basin hypolimnion of Clay Lake (see Fig. 17). Higher MeHg concentrations were also evident in the Wabigoon River downstream of Dryden when low flows could not assimilate organic loadings at Dryden and anoxic conditions developed (compare Fig. 3 and 4). During low-flow periods in 1980, dissolved oxygen values in much of the river were zero, while under normal flows there are measurable quantities of dissolved oxygen throughout the river (Parks et al. 1986).

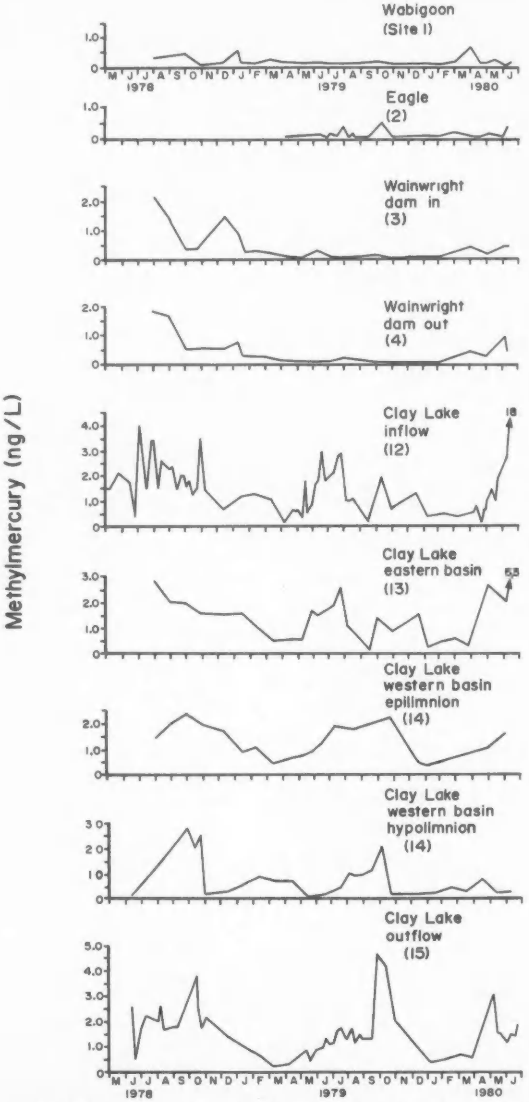

FIG. 4. MeHg concentrations (whole water) for selected sites in the Wabigoon River system, May 1979 – June 1980. Numbers in parentheses indicate the location of the sampling station as shown in Fig. 1. Note change in scale for ordinate axis for Clay Lake western basin hypolimnion.

Form of MeHg in Water

For all seasons and sites, little MeHg appeared to be associated with the operationally defined particulate phase (Table 5). Given that the analytical precision for MeHg is ±0.2 ng/L, it is generally impossible, in most cases, to distinguish between total MeHg and "soluble" MeHg as defined by field centrifugation.

Loadings

Loading calculations suggest that MeHg is being generated in the contaminated watercourse, particularly between Dryden

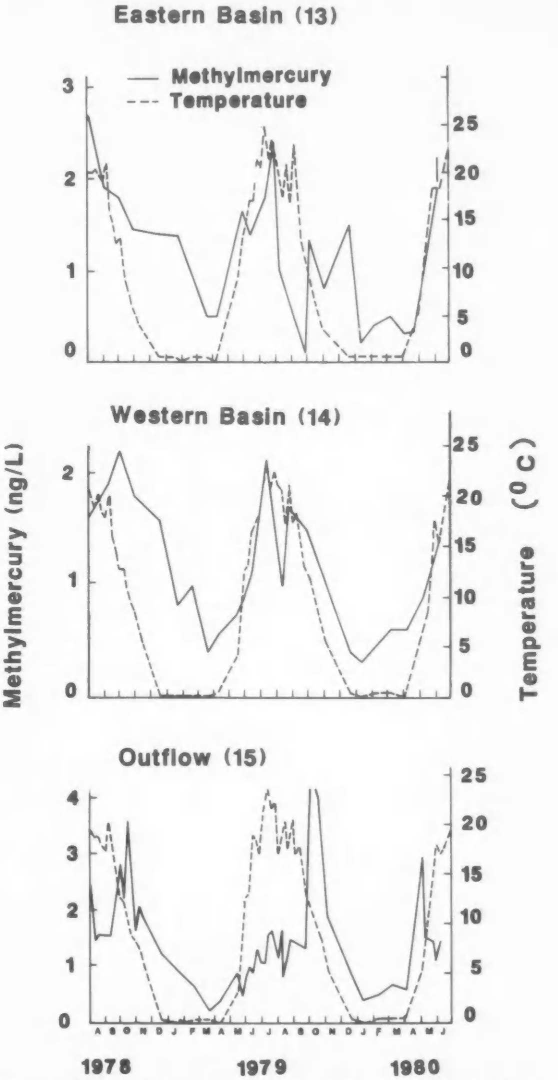

FIG. 5. MeHg and temperature at selected sites in Clay Lake, August 1978 – June 1980.

and the inflow to Clay Lake (Table 6). For the July 1979 – June 1980 period, the annual flux for the uncontaminated Wabigoon Lake outflow (Site 1) was about 0.05 kg. The annual loadings at the inflow and outflow of Wainwright Dam (Sites 3 and 4, respectively) were about 0.06 and 0.07 kg, respectively. Loadings increased at Minnitaki (Site 6) to 0.19 kg and at the inflow to Clay Lake (Site 12) to 1.1 kg. As a result, more MeHg was produced in the Wabigoon River between Minnitaki and Clay Lake than between Dryden and Minnitaki (Fig. 7). Loadings were unchanged from the inflow of Clay Lake to the inflow to Ball Lake. MeHg loadings from the Wabigoon River had no discernable effect on the English River system. Anthropogenically related MeHg flux from the Clay Lake inflow (Site 12) to Ball Lake was 83–86% of the total MeHg loading (Fig. 7).

Although there were no recurring temporal trends for MeHg loadings at either the inflow or outflow of Wainwright Dam, monthly loadings at these two sites were similar. This synchrony would be expected from the similarities in concentrations (Fig. 4) and flows. At both inflow and outflow of Clay Lake, monthly loadings were highest in the summer and lowest in the winter (Fig. 8). Daily loadings in June were similar to those during the spring runoff because the increased concentrations associated with higher water temperature compensated for the reduced flows (Fig. 9). The total MeHg load for the complete spring runoff was about 17% of the annual flux. Loadings at the outflow of Clay Lake followed the same general seasonal trends found at the inflow.

Net Production

For the entire contaminated Wabigoon River watercourse, minimum net anthropogenic MeHg production for the 1-yr period of July 1979 – June 1980 was 0.06 $\mu g \cdot m^{-2} \cdot d^{-1}$ assuming that MeHg was derived directly or indirectly from contaminated sediments.

Net production of MeHg on an annual basis within various reaches of the contaminated watercourse varied by orders of magnitude. The highest net production value (0.55 $\mu g \cdot m^{-2} \cdot d^{-1}$, observed in the Minnitaki to Clay Lake reach) was 100 times greater than the value for the Clay Lake to Ball Lake reach (Fig. 7). Increased production of MeHg was always evident within several days time-of-travel downstream of tributary junctions. After MeHg "stabilized" at a characteristic value for a particular sediment–water system, production decreased substantially. Within various geographical components of the Wabigoon system there were also seasonal differences in the production of MeHg. Minimum production of MeHg was about two times higher under ice-free conditions in the Dryden to Clay Lake reach in comparison with ice-covered conditions (Table 7).

Production was also higher during ice-free conditions in Clay Lake which contributed to seasonal changes in the MeHg content of Clay Lake waters (Fig. 2). Using data in Fig. 2 and loading data (Table 7), we calculated that during the 1978–79 ice-covered period there was a net loss of 173 g of MeHg from Clay Lake. In the following ice-free period, the minimum net MeHg production was 189 g or 0.037 $\mu g \cdot m^{-2} \cdot d^{-1}$. Losses during the 1979–80 ice-covered period amounted to 91 g.

Discussion

Reference site values of MeHg (Table 2; Fig. 3) are consistent with values for other systems of no known point sources of Hg (McLean et al. 1980). Because current ongoing industrial discharges of MeHg at Dryden did not increase concentrations downstream (Table 2; Fig. 4), elevated levels in the contaminated waters must result from in situ methylation processes. MeHg concentrations in well-mixed contaminated surface waters changed rapidly in response to changes in environmental conditions. One example is temperature (Fig. 5). Another example is provided by the time taken for uncontaminated waters to reach asymtotic MeHg concentrations when exposed to Hg-contaminated sediments.

Traditionally MeHg in water in contaminated systems is believed to have originated in sediments. And our results suggest that MeHg in water in the contaminated system can be described by sediment–water partitioning (Fig. 10). A preliminary model describing the sediment–water partitioning of MeHg can explain MeHg in water as being generated locally

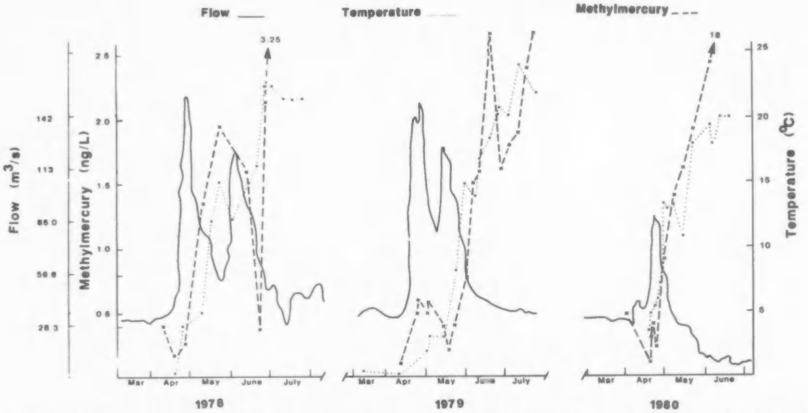

FIG. 6. MeHg, temperature, and flow data, inflow to Clay Lake (Site 12), during spring in 1978, 1979, and 1980.

Table 4. Correlations between methylation and temperature in surface waters of the Wabigoon River system, May 1978 – June 1980.

Site	Temperature		
	n	*r*	*p*
1. Wabigoon Lake	22	0.04	0.94
2. Eagle River	26	−0.06	0.79
3. Inflow to Wainwright Dam[a]	23	0.19	0.39
4. Outflow to Wainwright Dam[a]	23	0.46	<0.05
6. Minnitaki[a]	16	0.47	0.07
12. Clay Lake, inflow[a]	63	0.70	<0.001
13. Clay Lake, eastern basin[a]	23	0.75	<0.001
14. Clay Lake, western basin	22	0.64	<0.001
15. Clay Lake, outflow	47	0.27	<0.05
16. Inflow to Ball Lake	12	0.29	0.35

[a] When resuspension of bed sediments is not evident in water column. For river sites this is nonflood periods arbitrarily defined as flows <14 m³/s at Dryden and <56 m³/s at the inflow to Clay Lake (Parks et al. 1986). For Clay Lake eastern basin when total suspended solids <15 mg/L.

TABLE 5. Total MeHg and "soluble" MeHg concentrations (as defined by in situ centrifugation), Wabigoon River system, April–September 1979. April samples were collected during spring runoff from the unregulated watershed; waters from Wabigoon Lake and Eagle Lake watersheds were held for later power generation. May samples were collected during a severe flood created by controlled discharge at Dryden. July–September samples were collected during typical summer–fall discharges (Parks et al. 1986).

Site	Date	Flow (m³/s)	Total MeHg (ng/L) (influent)	"Soluble" MeHg (ng/L) (effluent)
1. Wabigoon Lake (control)	Apr. 27	15.1	0.3	<0.2
	May 12	90.1	<0.2	<0.2
	July 19	10.7	<0.2	0.3
	Sept. 20	14.0	0.2	0.2
2. Eagle River (control)	Apr. 25	15.5	<0.2	0.2
	May 11	17.6	<0.2	<0.2
	July 20	15.4	<0.2	<0.2
	Sept. 21	14.2	<0.2	<0.2
3. Wainwright Dam inflow	Apr. 24	15.3	<0.2	0.2
	May 9	31.2	0.3	<0.2
	Aug. 2	10.0	0.4	0.5
	Sept. 18	7.6	<0.2	<0.2
4. Wainwright Dam outflow	Apr. 28	15.3	<0.2	<0.2
	May 10	49.4	<0.2	0.2
	July 24	9.9	0.9	1.4
	Sept. 18	14.0	<0.2	0.2
6. Minnitaki	Apr. 25		0.4	0.3
	May 11		1.4	<0.2
	July 20		0.8	1.4
	Sept. 21		0.5	0.6
11. Quibell Falls	Apr. 26	148.9	0.9	0.5
	May 15	121.5	0.4	0.4
	July 23	32.6	2.2	2.1
	Sept. 25	25.2	12.0	15.0
15. Clay Lake outflow	Aug. 4	33.0	0.8	0.7

in sediments and imported from upstream. According to this model, once MeHg concentrations have "stabilized in any part of the contaminated system, MeHg exported downstream or lost to the atmosphere is balanced by releases from the sediment. For example, during the low-flow period in the fall of 1980, mean MeHg levels at contaminated river sites were unaffected by the duration of contact between sediment and water (Fig. 3). If time of contact had been important, concentrations should have increased downstream of Dryden, particularly in reaches which received little additional uncontaminated runoff. This approach is consistant with evidence for an equilibrium in sediments in another Hg-contaminated watercourse receiving woodwastes (Ottawa River, Kudo 1977) as well as sediments elsewhere (Bartlet and Craig 1981).

The similarity of concentrations among five "similar" sediment–water systems observed over a 6-wk period (Fig. 11) further supports that MeHg concentrations in waters were independent of residence time or time of contact with contaminated sediments. MeHg concentrations declined inside two 10-m-diameter sediment–water enclosures (Rudd et al. 1983) in the eastern basin of Clay Lake as did those outside at three nearby sites in the Wabigoon River and Clay Lake. The loss of MeHg with time from the water of these enclosures (Fig. 11) may have been due to losses to the atmosphere or, as will be discussed later, to demethylation in the water column. Likewise, the

TABLE 6. MeHg loadings (kg) for the Wabigoon River system for two 12-mo periods August 1978 – June 1980. The first period represents a typical water year; the second had very low flows in spring and summer. Sites 1, 2, and 17 are uncontaminated.

Site	August 1978 – July 1979[a]	July 1979 – June 1980[a]
1. Wabigoon Lake	0.09 (0.063–0.12)	0.05 (0.026–0.072)
2. Eagle River		0.07 (0.039–0.095)
3. Inflow to Wainwright Dam	0.29 (0.24–0.34)	0.06 (0.047–0.079)
4. Outflow to Wainwright Dam	0.22 (0.17–0.27)	0.07 (0.046–0.085)
6. Minnitaki		0.19
12. Inflow to Clay Lake	1.7	1.1
15. Outflow to Clay Lake	1.6	1.3
16. Inflow to Ball Lake		1.4
17. English River inflow to Ball Kake		3 (2.5–3.5)
18. English River outflow of Ball Lake		3 (2.3–3.7)

[a] >10% of MeHg results were below detection limits; therefore, upper and lower bounds on loadings are given. The upper one equates the "less than" value to 0.2 ng and the lower one equates it to 0.

reduction in MeHg concentrations and loadings in the Wainwright Reservoir (between Sites 4 and 5) during the summer and fall of 1980 (Fig. 3) can be ascribed to the same processes: volatization or demethylation. Settling of MeHg-enriched particulates at Site 5 from scoured upstream bed sediments (Jackson and Woychuk 1980) seems unlikely during the summer and fall of 1980. During this atypical low-flow period, flows were well below those required to resuspend bed sediments (Parks et al. 1986; Acres Consulting Services Ltd. 1983).

The average minimum net production value for this entire highly contaminated system (0.06 $\mu g \cdot m^{-2} \cdot d^{-1}$) is 100 times less than values produced in laboratory studies using fish as indicators of MeHg production and comparably contaminated sediments (Langley 1973; Akagi et al. 1979). In these studies, fish from a "clean" environment were placed in tubes containing contaminated sediments. MeHg production was measured by the quantity of MeHg taken up as fish "reequilibrated" with the new (contaminated) environment.

Sediment–water partitioning could also account for much of the variance in net production over the watercourse. The partitioning could explain, for example, why the Clay Lake to Ball Lake reach, which contains some of the most contaminated biota in the entire watercourse (Parks et al. 1984), has a MeHg net production value of less than 1% of that observed in other parts of the system and about 0.1% of the aforementioned laboratory results.

This disparity in production values supports the contention by Akagi et al. (1979) that net MeHg production is directly proportional to the rate that MeHg is removed from the sediment–water system. It would appear that laboratory studies which utilize biological "sinks" for MeHg in the water column can, to a certain extent, unwittingly adjust production simply by adjusting biomass to water ratios.

Our data suggest that sediment–water partitioning can be achieved within 1 d in the upstream portion of the river (Fig. 3) as was the case for total Hg in this system (Parks et al. 1986). In the upstream part of the system (Sites 1–4), during low flows, the highest mean MeHg concentrations in the entire system were

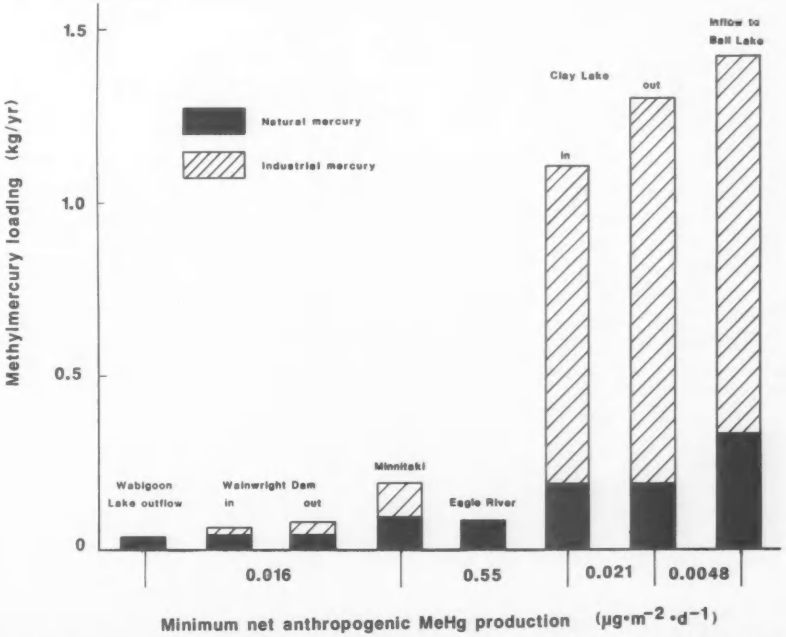

FIG. 7. Natural and anthropogenically related MeHg loadings at various sites in the Wabigoon River system, Dryden to Ball Lake, June 1979 – June 1980. See text for details on distinguishing between natural and anthropogenic sources.

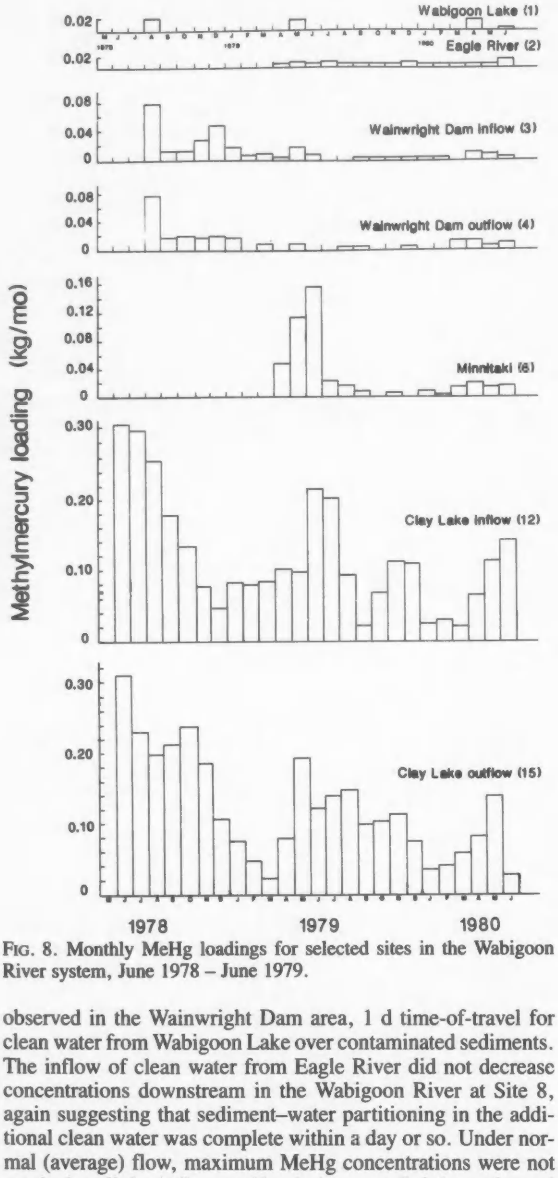

FIG. 8. Monthly MeHg loadings for selected sites in the Wabigoon River system, June 1978 – June 1979.

observed in the Wainwright Dam area, 1 d time-of-travel for clean water from Wabigoon Lake over contaminated sediments. The inflow of clean water from Eagle River did not decrease concentrations downstream in the Wabigoon River at Site 8, again suggesting that sediment–water partitioning in the additional clean water was complete within a day or so. Under normal (average) flow, maximum MeHg concentrations were not reached until the inflow to Clay Lake some 5 d time-of-travel downstream of Dryden. This may indicate that sediment–water partitioning of MeHg may take substantially longer during higher flows as one may expect sediment–water partitioning to be complete when MeHg concentrations in water "stabilized" at a maximum value. An alternate explanation could be that the biogeochemical characteristics of the watercourse downstream of Dryden, as influenced by industrial loadings, may favour lower MeHg concentrations at equilibrium under higher flows.

Evidence for a MeHg – Inorganic Hg Equilibrium in Water

MeHg in water can be described by sediment–water partitioning. However, we suggest that expanding the preliminary model to include methylation and demethylation in the water column (Fig. 12) explains the unusual geographic trend of these data, i.e. MeHg concentrations increase rather than decrease with distance from the point source. There are several lines of evidence to suggest that MeHg and inorganic Hg in most waters are at or approach equilibrium. There are relationships between MeHg and total Hg in the Wabigoon River system (Fig. 13; Table 8). In the ensuing discussion we use total Hg concentrations to approximate inorganic Hg levels because Hg is >90% inorganic in most cases (Table 8; Parks and Hamilton 1987). Generally, the fraction of total Hg present as MeHg increased with distance downstream of Dryden, most dramatically in the Dryden to Clay Lake reach. The pattern in site-specific variability in the fraction of total Hg that is in the methyl form suggests that the conditions governing the equilibrium change with distance downstream of Dryden.

There is a strong relationship between mean annual MeHg and total Mg concentrations for "similar" waters (Fig. 14). "Similar" waters are defined here as those which are well oxygenated and devoid of reduced sulphydral compounds discharged in pulp and paper mill wastes. Least squares regression for data in Fig. 14 yield an $r = 0.91$. Our linear fit to data in Fig. 14 is consistant with results of radiochemical studies by Rudd et al. (1983). They found a linear relationship between inorganic Hg concentrations and MeHg production over the range of inorganic Hg concentrations observed in our system.

There is no significant relationship between the MeHg to total Hg ratio and flow rate at several sites (Table 9), suggesting that the balance between the formation and degradation of MeHg is independent of residence time.

The ratio of MeHg to total Hg in water appears to be independent of time of contact with sediments. We examined the MeHg to total Hg ratio in surface waters in the dimictic western basin of Clay Lake. For the most part, contact of western basin surface waters with sediments occurs only during turnovers (Ontario Ministry of the Environment, unpubl. data) because of the steep shorelines (Parks et al. 1984). The MeHg to total Hg ratios associated with spring and fall turnover (Fig. 15) were similar to those in late summer and winter when surface waters had been isolated from direct contact with sediments for months.

The ratio between MeHg and inorganic Hg appears to be independent of loss rates to the atmosphere. Atmospheric losses of Hg should occur only under ice-free conditions. Thus, higher MeHg to total Hg ratios could be expected under ice-cover if an equilibrium did not exist and various Hg species continued to be released from the contaminated sediments. Again the Clay Lake data show no consistent evidence of an increasing MeHg to total Hg under ice-cover (Fig. 15).

The aforementioned evidence suggests that for many parts of the system MeHg to inorganic Hg ratio in water is independent of residence time, time of contact with sediments, and time of contact with the atmosphere. These characteristics are consistent with those of a MeHg – inorganic Hg equilibrium.

Apparent Kinetics

Two examples indicate that the MeHg to total Hg equilibrium can be established within days. During the low flows in the summer and fall of 1980, MeHg averaged 3.5% of total Hg in water at the first contaminated site, the inflow to Wainwright Dam (Site 4). This value is similar to the average of other downstream sites (excepting Wainwright Dam outflow, $x = 3.8$, range = 2.7–5.3, $n = 8$). Total Hg values used in calculations

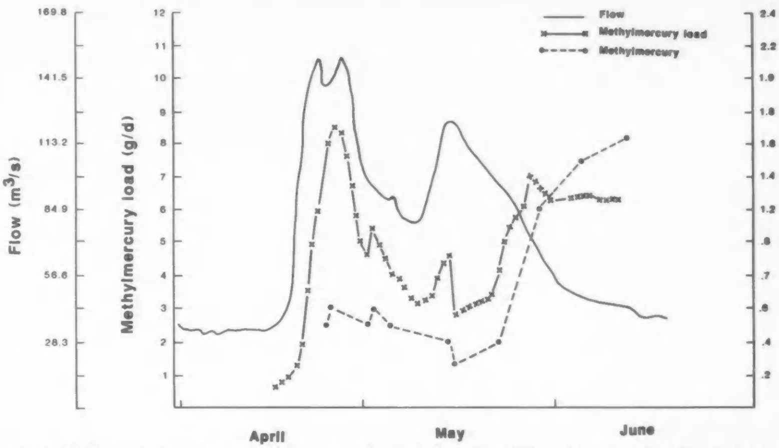

FIG. 9. MeHg concentrations, loadings, and flows during the 1979 spring runoff at Clay Lake inflow (Site 12).

TABLE 7. MeHg loadings (g) for the Wabigoon River, for ice-free and ice-covered periods, 1978–80.

Site	Ice-free (May–October)[a]	Ice-covered (November–April)[a]	
	1979	1978–1979	1979–1980
Wabigoon Lake outflow	31 (15–46)	37 (29–45)	16 (0–32)
Eagle River	52 (39–63)		22 (4–41)
Wainwright Dam inflow	46 (41–51)	133 (102–158)	39 (29–49)
Wainwright Dam outflow	29 (10–49)	79 (46–112)	41 (29–53)
Minnitaki	280		92 (78–106)
Clay Lake inflow	780	500	375
Clay Lake outflow	809	527	404
Clay Lake	+195	−215	−90
Ball Lake inflow	810	530	400

[a] >10% of MeHg results were below detection limits; therefore, upper and lower bounds on loadings are given. The upper one equates the "less than" value to 0.2 ng/L and the lower one equates it to 0.

are presented elsewhere (Parks et al. 1986). The time-of-travel to the Wainwright Dam is less than 1 d. The similarity between the ratio at the inflow to the Wainwright Dam and other sites support establishment of equilibrium within this period.

We have already shown that the MeHg to total Hg ratio is independent of flow rate at the inflow to Clay Lake. For those conditions the average time-of-travel (and hence residence time) was less than 5 d based on prevailing flows and time-of-travel data. Likewise, the MeHg to total Hg ratio stabilized within 2 d time-of-travel within the *river* during the 1980 spring runoff (Parks et al. 1984).

Factors Controlling the MeHg Concentrations at Equilibrium

We propose the following equation to as a framework for the discussion of variables affecting the MeHg – inorganic Hg equilibrium characteristics in water (and sediments). The equation is based on reviews of the methylation and demethylation processes (Beijer and Jernelov 1979; Bisogni 1979; Robinson and Tuovinen 1984) in conjunction with our field observations:

$$B \cdot \mathrm{Hg_{total}} \rightleftarrows \mathrm{MeHg_{total}} \cdot \alpha$$

where B = fraction of Hg available for methylation (commonly referred to as Hg^{2+} concentrations), $\mathrm{Hg_{total}}$ = total inorganic Hg, α = fraction of MeHg available for demethylation, and $\mathrm{MeHg_{total}}$ = total MeHg.

Both methylation and demethylation processes are believed to be primarily the result of microbially mediated processes (Jensen and Jernelov 1969; Craig and Moreton 1985; Ramlal et al. 1986).

We also expanded the model (Fig. 12) to include sediments where the vast majority of Hg in aquatic ecosystems is found (e.g. Ottawa River Project Group 1979; Jackson and Woychuk 1980). Due to the very rapid equilibrium reactions of various inorganic Hg and MeHg species (Stumm and Morgan 1981), both inorganic Hg and MeHg species probably partition within minutes between soluble and particulate phases in our system (Parks and Hamilton 1987).

In our system there is also probably an "equilibrium" between MeHg and inorganic Hg in sediments. This equilibrium is likely achieved even more rapidly than that proposed for the water column due to higher microbial activity, a key factor affecting the kinetics of the equilibrium reaction. The inferred time for equilibrium in our sediments is shorter than

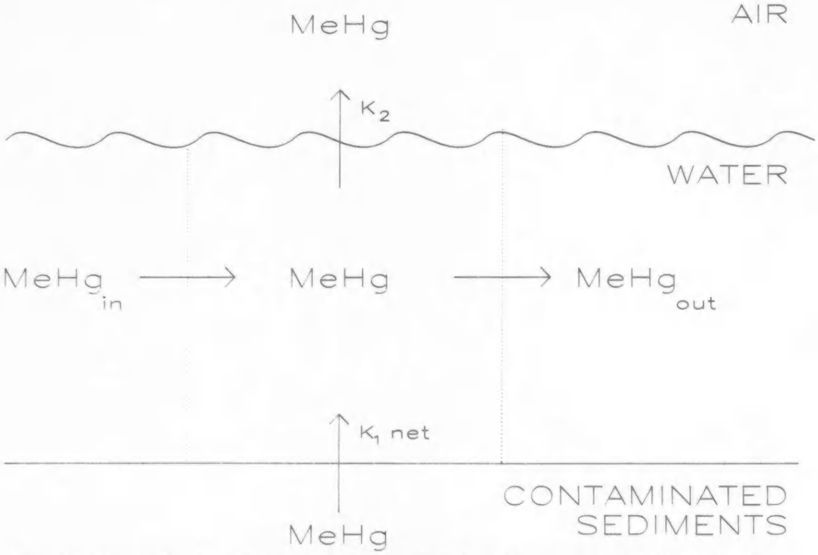

Fig. 10. Simple schematic diagram which can explain the sediment–water partitioning of MeHg.

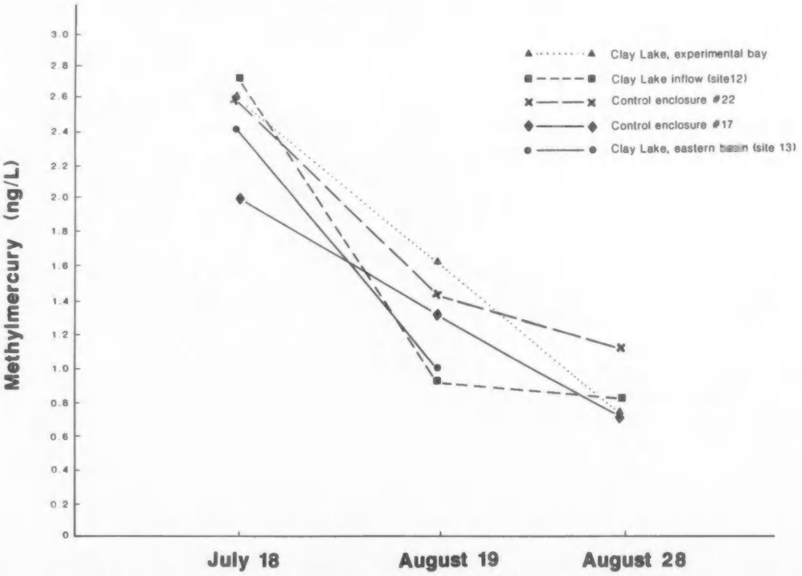

Fig. 11. MeHg concentrations at five locations in Clay Lake, July – August 1979. Enclosures were 10-m-diameter cylinders with flexible polyethylene walls which isolated sediments and about 1.5 m of overlying water in the experimental bay in Clay Lake eastern basin (near Site 13) (Rudd et al. 1983).

that observed for MeHg concentrations to stabilize after the addition of inorganic Hg to other sediments. In these studies (Jensen and Jernelov 1969; Olson and Cooper 1976), equilibrium can be argued to be completed in approximately 10 d.

The short time frame for equilibrium proposed here is consistent with systems in which bacteria have been previously exposed to high Hg levels. Wollast et al. (1978) showed that equilibrium is most rapid when bacteria have been exposed to high inorganic Hg and MeHg levels, as only the most inorganic Hg- and MeHg-resistant bacteria will remain in the microbial community. Application of methylation and demthylation rates derived from indigenous acclimated bacteria (Wollast et al. 1978; Spangler et al. 1973) to conditions in our system would result in equilibrium being achieved in 1 d. The most Hg-resistant species in the Wabigoon system have had ample time to establish themselves prior to our studies, over 15 yr. Thus, our

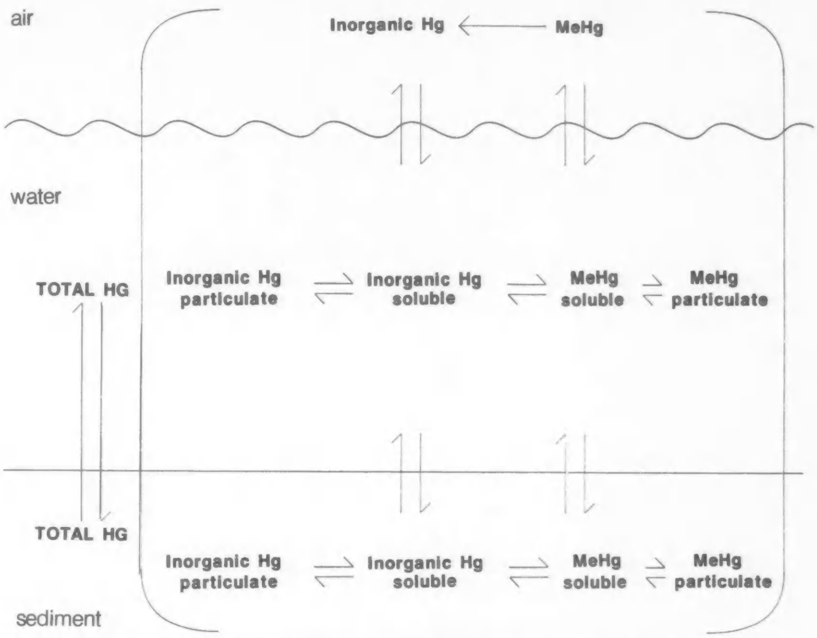

FIG. 12. Schematic diagram of key processes governing MeHg concentrations in water.

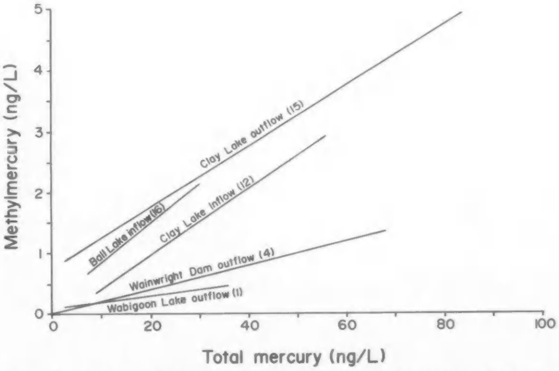

FIG. 13. Relationship between MeHg and total Hg at selected sites in the Wabigoon River system in the absence of scoured bed sediments, August 1978 – June 1980. Statistics for linear relationships are presented in Table 8.

TABLE 8. MeHg – total Hg relationships for water in the Wabigoon River system. Values have been excluded when bottom sediments were scoured.

Site	Regression equation	n	r	P
1. Wabigoon Lake	$y = 0.010x + 0.11$	24	0.63	<0.001
4. Wainwright Dam inflow	$y = 0.022x - 0.10$	21	0.62	<0.01
5. Wainwright Dam outflow	$y = 0.019x + 0.03$	21	0.61	<0.01
7. Caly Lake inflow	$y = 0.054x - 0.12$	48	0.66	<0.001
8. Clay Lake, eastern basin	$y = 0.055x + 0.11$	18	0.58	<0.05
9. Clay Lake, western basin	$y = 0.052x + 0.40$	18	0.51	<0.05
10. Clay Lake outflow	$y = 0.049x + 0.76$	53	0.42	<0.001
11. Wabigoon River, inflow to Ball Lake	$y = 0.064x + 0.21$	10	0.72	<0.05

very rapid suggested time frame for equilibrium (on the order of days) is consistent with the known chemistry and microbiology of Hg-contaminated systems.

Many of the variables which affect this MeHg – inorganic Hg relationship in water fluctuated substantially on a seasonal and/or geographical basis in response to changing hydrological and limnological conditions and waste loadings introduced at Dryden. We briefly examine these variables below.

Inorganic (essentially total) Hg in the surface waters of the Wabigoon/English River system are derived from both point and diffuse sources (Parks and Hamilton 1987; Parks et al. 1986; Jackson et al. 1982). Inorganic Hg concentrations in the watercourse are best described by sediment water – suspended solids partition coefficients (Parks et al. 1986). At control sites, inorganic Hg is presumably at sediment–water–air steady state.

Point source loadings from the pulp and paper mill at Dryden, appear to directly affect concentrations only in waters immediately downstream of Dryden (i.e., Wainwright Dam) (Parks et al. 1986). At contaminated sites farther downstream inorganic mercury concentrations in waters can be affected by mercury in surface sediments, organic content of sediment, suspended solids concentrations, anoxia, temperature and lake stratification (Parks et al. 1980, 1984, 1986; Parks and Hamilton 1987, Jackson et al. 1982).

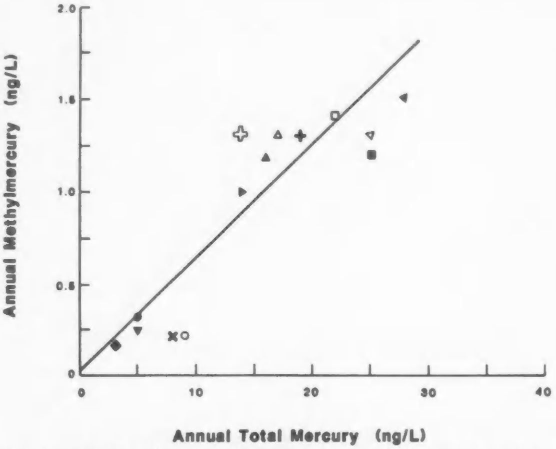

FIG. 14. Relationship between MeHg and total Hg in water at selected sites in the Wabigoon/English River system for two consecutive study periods of 12 mo. Open symbols: first study year, August 1978 – July 1979; closed symbols: second study year, July 1979 – June 1980. ○●. Wabigoon Lake; ◆, Eagle River; □■, Clay Lake eastern basin; △▲, Clay Lake western basin; + +, Wabigoon River at Clay Lake outflow; ??, Wabigoon River at Ball Lake inflow; ▶, Wabigoon River at Ball Lake inflow; ◀, English River upstream of Ball Lake; ×, English River at Ball Lake outflow.

TABLE 9. Correlation between MeHg to total Hg ratio and flow for selected sites in the Wabigoon River system in the absence of scoured bed sediments and when both MeHg and total Hg were measurable.

Site	*r*	*n*	*P*
Wabigoon Lake outflow	0.20	8	>0.05
Clay Lake inflow	0.17	46	>0.05
Clay Lake outflow	0.11	52	>0.05

The fraction of inorganic Hg available for methylation, *B*, is determined by the various factors governing Hg speciation and in particular the solid-solution partitioning, i.e. pE, pH, and type and concentrations of complexing inorganic and organic ligands and chelating agents. The environmental chemistry of Hg^{2+} is dominated by reactions with reduced sulphur, chlorides, hydroxides, and suspended solids (Stumm and Morgan 1981), all factors important in the Wabigoon/English River system (Parks et al. 1986; Jackson et al. 1982; Jackson and Woychuk 1980; Rudd and Turner 1983; Parks 1976; Armstrong and Hamilton 1973). These factors fluctuate seasonally and geographically due in part to the natural cycling of these constituents but as well as in response to the anthropogenic effects loadings. Typically 25–50% of the Hg is associated with operationally defined particulate matter (Parks et al. 1986; Rudd and Turner 1983; Armstrong and Hamilton 1973); however, this percentage, and hence *B*, can change with the resuspension of bed sediments.

Changes in *B* probably arise because the Hg^{2+} fraction is part of the soluble Hg pool, and one would not expect increases in methylation rates during periods of resuspended bed sediments, at least not in uncontaminated systems, because although total Hg concentrations in water may rise dramatically, dissolved Hg (and hence Hg^{2+}) concentrations do not (Lindberg and Harris 1977; reviewed in Parks and Hamilton 1987).

Little change in soluble Hg concentrations during sediment resuspension may simply be the result of bed sediments having "equilibrated" with soluble Hg in the water column prior to deposition. In this case the soluble Hg pool (and Hg^{2+} fraction) in the water column can be viewed as a constant. Consequently *B* will be inversely correlated with the quantity of Hg present in the suspended form.

We examined, indirectly, the effects of resuspending bed sediments at the inflow to Clay Lake on the MeHg – inorganic Hg equilibrium. Monthly MeHg and total Hg data for the first study year were plotted for the Wabigoon inflow to Clay Lake (Fig. 16). The data point for the spring runoff, when total Hg in the water increased much more sharply because of resuspension of river bed sediments (Parks et al. 1986; Jackson et al. 1982), is an outlier. A parallel phenomenon was observed for the soluble – particulate Hg relationship under the same conditions (Parks et al. 1986). These data suggest that a much larger fraction of the total Hg in the water during spring runoff is not immediately available for methylation in comparison with other times of the year.

There is evidence to suggest that *B* may correlate with total Hg concentrations in contaminated water under certain conditions. Seasonal increases in total Hg in contaminated waters (Parks and Hamilton 1987; Parks et al. 1986) have been attributed in part to changes in the supply of soluble Hg from sediment (Parks et al. 1986). Both field and limnocorral studies indicate that increasing the supply of soluble inorganic Hg to waters will result in an increased amount of Hg available for methylation (Parks and Hamilton 1987; Rudd et al. 1983) and consequently higher concentrations of MeHg.

Using solubility alone as an indicator of the amount of inorganic Hg and MeHg available for bacteria transformation, it would appear from our field studies that α is greater than *B*. For pH and Cl levels encountered in our system, much of the MeHg, like inorganic Hg, may be bound to hydroxides and chlorides (Stumm and Morgan 1981) in well-aerated surface waters.

Another factor which strongly affects *B* is the absence or presence of reduced sulphur in waters. Binding preferences will shift dramatically as oxygen levels and pE fall and reduced sulfur compounds appear as Hg binds much more strongly with reduced sulphur compounds (Baughman et al. 1973). When reduced sulphur compounds and thiols are present, such as in most sediments, the hypolimnion of Clay Lake, or the Wabigoon River receiving pulp and paper mill organic wastes, the Hg^{2+} concentrations can be dramatically reduced (probably by orders of magnitude) due to the very high affirmity of Hg^{2+} for S^{2-}, $R\text{–}S^{-}$, and R–SH compounds (Stumm and Morgan 1981). In other sediment water studies, Fagerstrom and Jernelov 1971) observed that systems containing sulphide produced MeHg at a rate 1000 times less than that for systems in which sulphide was absent.

Much of the unknown variance in the MeHg – total Hg relationships (Fig. 13; Table 8) could be explained by microbial activity. Increases in MeHg production in sediments and waters of this system by the stimulation of microbial populations have already been demonstrated (Furutani and Rudd 1980). Additionally, elevated MeHg levels in another polluted river–lake system have also been largely ascribed to the activities of Hg-methylating microorganisms (Jackson 1986).

Microbial activity has increased downstream of Dryden as a result of anthropogenic discharges of phosphorus and organic wastes. As a result of these organic loadings, microbial populations have increased. Counts of certain bacteria (e.g. total coliform) are 10^3–10^4 times higher than reference site values

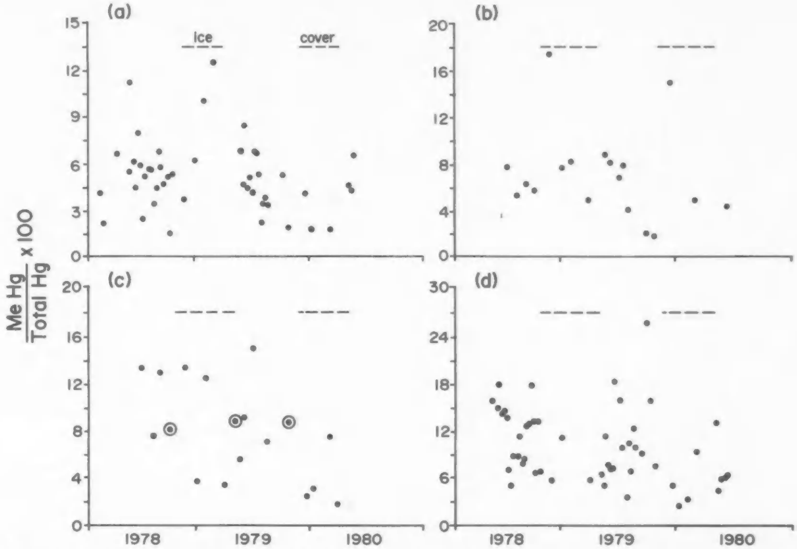

FIG. 15. Scattergram of MeHg to total Hg ratio and time for Clay Lake sites, June 1978 – June 1980. Circled data points in the western basin (Site 14) represent times of complete lake mixing at spring and fall turnovers.

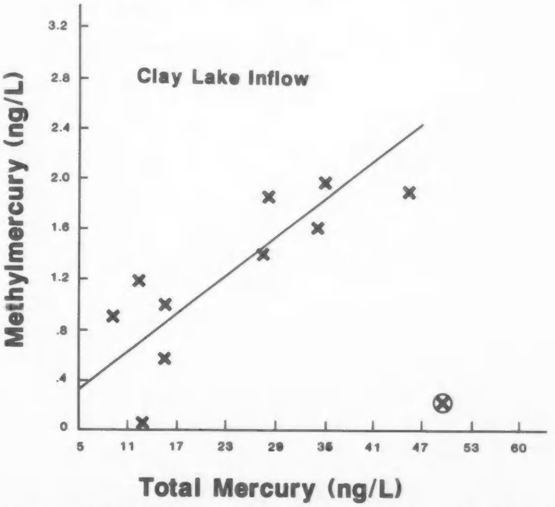

FIG. 16. Relationship between MeHg and total Hg from data collected monthly during the first study year at Clay Lake inflow. The regression line is drawn omitting the circled value datum which occurred during spring flood when much more of the organic Hg was in the suspended form and unavailable for methylation.

(Ontario Ministry of the Environment 1981). There were also increases in chlorophyll *a* in Clay Lake in comparison with Wabigoon Lake, suggesting that there is more organic matter and hence microbial activity in Clay Lake. This is largely attributed to upstream anthorpogenic phosphorus loadings (Parks et al. 1984). Increases in microbial activity in Clay Lake will have helped shift the MeHg – inorganic Hg equilibrium to higher concentrations of MeHg in contaminated waters at equilibrium in comparison with reference sites (Fig. 13). This interpretation is supported by studies by Wright and Hamilton (1982) who used microbasins (mini sediment–water flow-through systems) to study the kinetics of the overall methylation process. Using Wabigoon River sediments in their systems, they reported the most increase in MeHg concentrations after adding readily metabolized organic material to water.

Effects of Flow, Temperature, Anoxia, and Other Chemical Factors

In the upper Wabigoon River between Dryden and Minnitaki, both low and high flows influenced MeHg levels. Under low flows, when the river turned anoxic, higher MeHg concentrations were evident.

The effects of high flows on MeHg concentrations appear to be site specific and dependent to some extent on the quantity of bed sediments which were resuspended. In general, high flows did not greatly increase MeHg concentrations at most sites. In the Wainwright Dam area, MeHg concentrations remained very low even during the severe flood conditions in May which increased total Hg concentrations an order of magnitude as a result of resuspended bed sediments (Jackson et al. 1982; Parks et al. 1986). Evidently the methylating environment in the Wainwright Dam area is very poor.

At Minnitaki only were the highest MeHg concentrations observed during the severe flood in May. At this time, almost all the MeHg was associated with suspended sediments. Detailed hydrological records are not available for Minnitaki; however, we observed that flood levels in May, during controlled discharges, were similar to those of elevated discharges in April from unregulated runoff. As MeHg concentrations in April were much lower than those in May, it is apparent that the source of flow, as well as flow magnitude, is an important variable governing MeHg concentrations during flood events. At Quibell, flood events did not affect MeHg concentrations as much as other seasonal factors.

It appears that the MeHg – inorganic Hg equilibrium changes under anoxia (Fig. 17), although the exact cause(s) for elevated

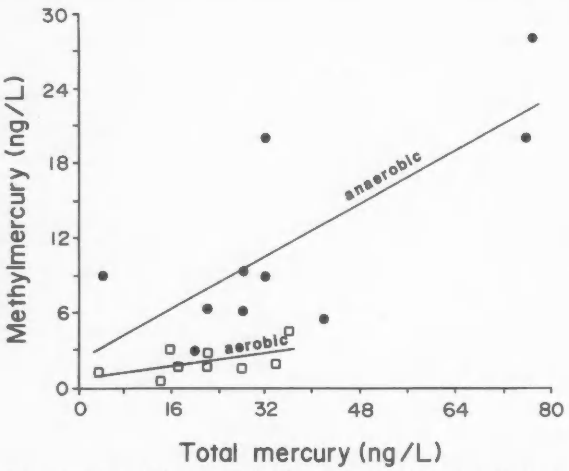

FIG. 17. Relationship between MeHg and total Hg in the hypolimnion of Clay Lake (Site 14) with oxygen present ($r = 0.57$, $n = 9$, $0.05 < p < 0.01$) and without oxygen ($r = 0.76$, $n = 10$, $p < 0.01$).

MeHg concentrations remains to be defined. The absence of oxygen can lead to changes in Hg speciation, microbial activity, and ligands for both organic and inorganic Hg. All these factors can affect MeHg concentration at equilibrium.

Although there is evidence to show that MeHg concentrations increase with temperature, the relative importance of a number of possible factors, including enhanced solubilities, increased microbial activity, and bioturbation, remains to be discerned. It would appear that in some cases, temperature-dependent factors governing the flux of Hg from sediments to waters may be much more important than the effect of increasing temperature on methylation rates per se. Multilinear regressions of MeHg on total Hg and temperature in the water column suggest that in the presence of a MeHg – inorganic Hg equilibrium, temperature is not the major factor affecting MeHg levels at most sites (Parks et al. 1984).

Several water quality variables (including total phosphorus and pH) are correlated with MeHg concentrations at some but not necessarily all surface water sites (Parks et al. 1984). A discussion of the importance of these variables on MeHg concentrations or the MeHg – inorganic Hg equilibrium is beyond the scope of this paper but will be addressed in a future paper.

Causes of Regional Variation in MeHg Concentrations

Thus far we have attempted to shed light on a number of individual factors which can affect MeHg concentrations in various waters. Here we offer explanations for an unusual geographical trend; for well-aerated waters, MeHg concentrations do not decrease with distance from the original point source of Hg but actually increase, particularly between Dryden and Clay Lake. We believe that the basis of this trend lies with the fraction of Hg available within the watercourse for methylation. On the basis of total inorganic Hg supply, one would not expect MeHg concentrations to increase downstream (Parks et al. 1986). And as previously discussed, microbial activity in water decreases with distance downstream as the soluble organic wastes are metabolized. Likewise, microbial activity in surface sediments is also expected to decrease with distance downstream as carbon concentrations in sediment decrease (Parks et al. 1984). On the basis of total Hg concentrations and microbiology, highest MeHg concentrations would be expected near Dryden. As MeHg concentrations in water are lowest here, we are left to conclude that the inorganic Hg available here for methylation is much lower than at sites farther downstream. Both sediments and waters immediately downstream of Dryden have high concentrations of reduced sulphur compounds (Parks 1976; Parks et al. 1986; Jackson et al. 1982). The presence of such compounds would dramatically reduce Hg available for methylation. As the receiving stream assimilated the organic wastes and sulphide was removed from waters, more of the Hg would become available for methylation.

Source of MeHg in Water: Water or Sediment

MeHg in contaminated waters can be derived from production in the water column, resuspension of MeHg-enriched sediments, and from the release of soluble MeHg from sediments. The importance of these various sources at any given site to MeHg levels in waters depends on processes which govern the flux of soluble and particulate MeHg species from sediments to waters and vice versa along with those factors governing the MeHg equilibrium concentrations in each compartment.

As previously mentioned it is generally believed that most MeHg in waters is generated in contaminated surface sediments owing to higher inorganic Hg concentrations and microbial activity. There are, however, several lines of evidence which do suggest that methylation and demethylation of mercury *in waters* (as opposed to sediments) in our system plays a very important role in governing MeHg concentrations in this system.

(1) Fish in limnocorrals isolated from sediments accumulated 20 times more radiolabelled Hg than fish in limnocorrals in contract with the sediments (Rudd and Turner 1983). Because a portion of the radiolabelled Hg in fish in these studies is in the methyl form (Rudd and Turner 1983) and because water is the source of the majority of Hg bodyburden in fish, the biological data suggest that the inorganic Hg does not have to be cycled through sediments for significant methylation to occur.

(2) While atmospheric losses of MeHg could explain losses of MeHg from the water column in both the river and lake enclosures, demethylation in the water column could also be invoked. Under ice-cover, when atmospheric losses of MeHg would be nil, the seasonal decrease in MeHg concentrations in the eastern basin of Clay Lake could be largely due to demethylation processes. Our field studies indicate that much more of the MeHg than inorganic Hg is in the "soluble" state and hence the loss of inorganic Hg from waters to sediments is likely significantly faster than MeHg. However, the equilibrium value (K) for MeHg does not change over the winter, despite the reduction in total Hg lost from the water column, suggesting that MeHg was probably demethylated in water.

(3) If we have made our case that MeHg in waters are at equilibrium, demethylation must occur in the water column because K values in contaminated waters are expected to be lower than values for surficial aerated contaminated sediments. The magnitude of the increase in K in surface sediments remains to be established. However, given the characteristics of, say, the eastern basin of Clay Lake (discussion following) and the Bisogni (1979) equation dealing with MeHg production, an increase in K of at least 2 or 3 in surficial sediments would not be unexpected. In the eastern basin of Clay Lake, Hg^{2+} concentrations in the aerated surface sediments would be expected

to be much higher (approximately five times) than overlying waters based on the known geochemistry of Hg, the sediment profiles for Hg (Parks and Hamilton 1987), sediment–porewater partitioning of Hg in clay-rich sediments for this system (Jackson and Woychuk 1980), soluble–particulate partitioning of Hg in the water column (Parks et al. 1986; Rudd and Turner 1983), and other biogeochemical characteristics of sediments such as pH and pE (Jackson and Woychuk 1980) and waters (Parks et al. 1984). Microbial activity would also be expected to be higher in surface sediments. Increased microbial activity and increased Hg^{2+} concentrations should lead to higher K values *at equilibrium* in surface sediments in comparison with overlying waters. Our observations that the MeHg equlibrium value in the eastern basin of Clay Lake is (1) independent of losses to the atmosphere (under ice-cover), (2) independent of residence time, and (3) displays a K value in water which is expected to be much different (lower) than expected in sediment porewaters suggest that demethylation in water is an important process. These observations are particularly significant for the eastern basin because this site has characteristics which are most favourable for the generation and transport of MeHg from sediments to overlying waters, i.e. sediments with high Hg concentrations, an aerated sediment–water interface, a large and varied benthic community, and high productivity.

(4) Methylation of Hg in waters (as compared with sediments) should be enhanced where microbiological actively in the water column is stimulated preferentially in comparison with surface sediments. Such conditions existed in the Wabigoon River downstream of Dryden when large quantities of soluble biodegradable wastes were discharged into the water column without treatment. For example, BOD_5 levels downstream of the mill averaged 28 mg/L as compared with 0.88 mg/L upstream (Parks 1976). Total coliform bacteria counts indicated that at least one of the components of the bacterial community was significantly stimulated (Parks 1976; Ontario Ministry of the Environment 1981).

(5) Methylation in water (versus sediments) would be favoured at our reference (control) sites in comparison with contaminated stations because the concentration gradient between MeHg in sediment porewaters and overlying lake waters would be lower than at contaminated sites. Hg^{2+} concentrations in well-aerated uncontaminated surface waters are expected to be at least as high or higher than those in porewaters of surface sediments, as highest Hg^{2+} concentrations can be expected at higher redox values (Stumm and Morgan 1981). Studies on the solid-solution chemistry of Hg in waters and sediments in this system provide supporting evidence (Jackson et al. 1982; Parks et al. 1986; Parks and Hamilton 1987). Studies on another shield watercourse also provide supporting evidence in that agitated, well-aerated water tends to increase the availability of inorganic Hg for methylation (Jackson 1986). Methylating and demethylating bacteria are common in nature (Robinson and Tuovinen 1984) and Hg^{2+} concentrations in uncontaminated waters are comparable with those in sediment porewaters, so the question of where MeHg in waters is derived, sediments or the water column, may be reduced to the relative importance of microbial activity in waters versus sediments. Studies in other Canadian Shield lakes indicate that *both* waters and sediments are important compartments for the breakdown of organic material (J. W. M. Rudd, pers. comm.). Similar conclusions have been reached in Europe (Schwoerbel 1987). The relative importance of microbial activity in the water column increases with lake depth, as organic material takes longer to reach the sediments and hence is more likely to be broken down by bacteria. Eagle Lake, an upstream uncontaminated water body, has basins approximately twice the depth of those lakes directly on the Wabigoon River system. Consequently, in Eagle Lake microbial methylation/demethylation in uncontaminated waters is likely more important than in waters on the contaminated system.

On a watercourse basis, if the residence time of soluble Hg in waters and/or sediments is longer than the time required to achieve equilibrium, then equilibrium processes should largely govern MeHg concentrations in that compartment. As previously discussed, the time to achieve equilibrium for given environmental conditions in either compartment in much of our system appears to be on the order of days.

We can gain a perspective on the residence time of Hg in waters and sediments from our observation that Hg levels in the Wabigoon River system are best described by a sediment–water partition (Parks et al. 1986) coupled with our understanding of Hg fluxes from sediments to waters (Parks et al. 1986; Parks and Hamilton 1987) and waters to sediment (Rudd and Turner 1983; Parks et al. 1986). For typical hydrological conditions, sediment–water partitioning is complete in almost all of the contaminated river–lake system (Parks and Hamilton 1987; Parks et al. 1984, 1986). Sediment–water partitioning is incomplete only in the immediate vicinity of the junction of uncontaminated tributaries (i.e. Eagle River) where clean waters meet contaminated river sediments.

For partitioned systems, estimates of residence times in the water column can be obtained by the numerous radiochemical studies in Clay Lake (Rudd et al. 1983). Although these studies largely measured *net* losses to the sediments, the values are similar to those observed for the epilimnion of other shield lakes in which lake stratification prevented significant feedback of radiolabelled Hg lost to the hypolimnion.

Because the rates of Hg going to the atmosphere are believed small compared with the rates of Hg cycled through sediments from water for this system (Parks and Hamilton 1987) and because these waters are best described by sediment–water partitions (Parks et al. 1986), it is self-evident that the flux of soluble Hg from contaminated sediments to waters would, at a minimum, be similar to that observed for the flux from water to sediment, i.e. on the order of a week or week(s).

Thus, for much of the watercourse it appears that the residence time of soluble Hg in the water column exceeds the time sufficient for water column chemical and biological processes to govern MeHg levels.

There are locations in our system, however, when essentially all MeHg in the water column is probably derived directly from sediments. During high flows when MeHg-enriched bed sediments are temporarily resuspended (i.e. Minnitaki) is one example. Another condition involves the flux of MeHg from sediments to waters at the junction of tributaries where clean waters enter the contaminated watercourse. At these locations the Hg flux from sediments to waters is much greater than that observed at downstream sites where sediment–water partitioning is complete (Parks et al. 1986; Parks and Hamilton 1987). Under these conditions, anthropogenic MeHg in the water is probably derived directly from contaminated sediments.

Net Production in Clay Lake

The MeHg content of Clay Lake waters (Fig. 2) likely gives a good approximation of net production trends for comparable

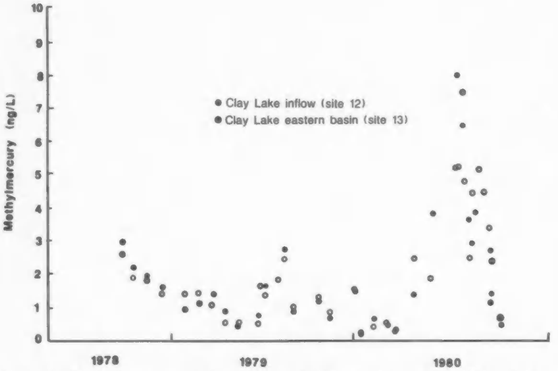

FIG. 18. MeHg concentrations for the inflow to Clay Lake (●) and the eastern basin of Clay Lake (○), August 1978 – November 1980.

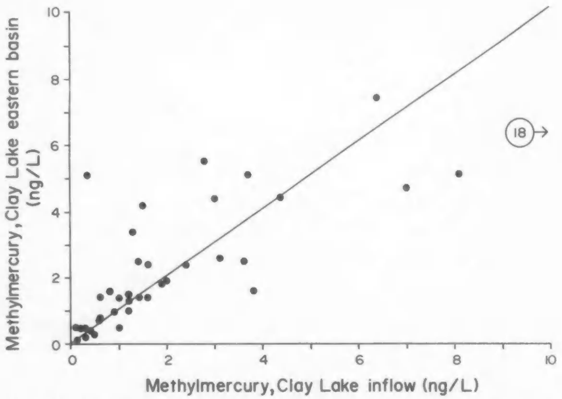

FIG. 19. Scattergram of MeHg concentrations at Clay Lake inflow versus Clay Lake eastern basin, August 1978 – November 1980. Excluding an outlier ($r = 0.74$, $n = 38$, $p < 0.01$), 1:1 line represents equal concentrations at both sites.

water years because (1) of the similarity in loadings entering the leaving Clay Lake for several periods (Table 7) and (2) the time for MeHg to "partition" between sediments and water is much less than the theoretical residence time for Clay Lake waters. Under normal hydrological regimes, net seasonal production of MeHg during ice-free conditions can be expected to decrease continuously with time until contaminated substrate is buried and the lake is restored to natural conditions. This process will likely take many decades according to recovery trends for several different organisms (Parks and Hamilton 1987). Also as a result of seasonal fluctuations in MeHg production, annual net MeHg production in uncontaminated lakes may well approach zero if net atmospheric exchange of MeHg is not a significant process affecting MeHg concentrations in waters.

Considerations in the Design of a Remedial Program

An understanding of factors governing MeHg concentrations, speciation, and production in contaminated systems yields insight into the effectiveness of a suite of ameliorative proposals.

The Ontario Ministry of the Environment has identified ameliorative actions that reduce mental and physical health risks to the people of the Grassy Narrows and Whitedog communities (Donnan 1986), and traditional approaches (i.e. dredging) to restore the watercourse were discussed by Acres Consulting Service Ltd. (1983).

Our results clearly suggest that for any given set of environmental conditions, MeHg concentrations in waters at any given site would "stabilize" within days. The rapidity with which MeHg concentrations in water appear to respond to environmental changes suggests that MeHg concentrations are largely the result of "local" environmental variables. Evidence that MeHg is locally generated is shown by data for the inflow and eastern basin of Clay Lake (Fig. 18). MeHg trends at both sites are similar, with no suggestions of the 11-d lag that would be expected if MeHg in the eastern basin was derived solely from the inflow of the Wabigoon River. The relationship between MeHg concentrations at both sites is adequately described by a linear relationship (Fig. 19), despite flow variations of over an order of magnitude. These results suggest that MeHg concentrations at both sampling sites are largely governed by similar processes and that concentrations in the eastern basin could have remained the same even if there was no MeHg load entering Clay Lake from the Wabigoon River.

Therefore, MeHg production in the most contaminated part of the Wabigoon River system, Dryden to Minnitaki, as defined by contamination in sediments, has very little impact on Clay Lake, the first viable fishery downstream of Dryden. Thus, even if the Dryden to Minnitaki section were completely restored, it would likely have only marginal effects on Clay Lake and probably no effect on the English River system. The large reservoirs of anthropogenic Hg currently in sediments in downstream reaches are not now a net source for overlying waters but will become so if Hg concentrations in upstream reaches are reduced (Parks and Hamilton 1987).

Innovative, procedures are believed to be more cost effective discussed elsewhere (Rudd et al. 1983; Allan et al. 1984; Parks and Hamilton 1987). These procedures are primarily designed to reduce the fraction of inorganic Hg available for methylation (lowering B) and can be instituted at any site.

Summary, Conclusions, and Future Research

(1) Ten years after major loadings of inorganic Hg from a chloralkalai plant were sharply reduced, MeHg levels in waters in most of the Wabigoon River system downstream of Dryden, Ont., remain markedly elevated. The pulp and paper complex at Dryden (Great Lakes Forest Products Ltd.) does not at present (1979–82) directly contribute significant quantities of MeHg to the Wabigoon River. MeHg concentrations are the result of in situ methylation processes.

(2) MeHg concentrations in well-mixed surface waters responded rapidly (within days) to new environmental conditions. Thus, "local" environmental variables govern MeHg concentrations in lakes and slow-moving rivers.

(3) MeHg concentrations in surface waters frequently fluctuate an order of magnitude within 1 yr at many sites. MeHg concentrations were positively related with temperature at many sites.

(4) MeHg concentrations increased substantially in the hypolimnion of Clay Lake during stratification and subsequent anoxia. Concentrations could exceed those in the epilimnion contributing to noticeable increases in concentrations in surface waters after lake turnover, especially in the fall.

(5) MeHg appears to be in equilibrium with inorganic Hg in water. The percentage of total Hg in the methyl form increased from Dryden to Clay Lake. This counterintuitive result was ascribed to an increase in the amount of Hg available for methylation.

Evidence of a MeHg – inorganic Hg equilibrium in water suggests that future research on Hg problems could well benefit from a shift from sediments to sediment–water systems, with investigation of the balance between methylation and demethylation in the water column.

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References

ACRES CONSULTING SERVICES LTD. 1983. Studies on moving sediments in the Wabigoon River for the Ontario Ministry of the Environment, 1982. 121 p. (Available from the Ontario Ministry of the Environment, Thunder Bay, Ont.)

AKAGI, H., D. C. MORTIMER, AND D. R. MILLER. 1979. Mercury methylation and partition in aquatic systems. Bull. Environ. Contam. Toxicol. 23: 372–376.

ALLAN, R. J., T. BRYDGES, D. DODGE, R. D. HAMILTON, D. G. JEFFS, AND K. SHIKAZE. 1984. Mercury pollution in the Wabigoon/English River system of northwestern Ontario and possible remedial measures. Final Rep. of the Steering Committee. Vol. 1 and 2. Published by Government of Canada (Dep. of Environment) and Government of Ontario (Ministry of the Environment). 570 p.

ARMSTRONG, F. A. J., AND A. L. HAMILTON. 1973. Pathways of mercury in a polluted northwestern Ontario lake, p. 131–156. *In* P. C. Singer [ed.] Trace metals and metal–organic interactions in natural waters. Ann Arbor Science Publishers, Ann Arbor, MI.

BARTLET, P. D., AND P. J. CRAIG. 1981. Total mercury and methyl-mercury levels in British estuarine sediments — II. Water Res. 15: 34–47.

BAUGHMAN, G. L., J. A. GORDON, N. L. WOLFE, AND R. G. ZEPP. 1973. Chemistry of organomercurials in aquatic systems. E.P.A.-66013-73-012.

BEIJER, K., AND A. JERNELOV. 1979. Methylation of mercury in aquatic environments, p. 203–210. *In* J. O. Nriagu [ed.] The biogeochemistry of mercury in the environment. Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands.

BISOGNI, J. J. 1979. Kinetics of methylmercury formation and decomposition in aquatic environments, p. 211–230. *In* J. O. Nriagu [ed.] The biogeochemistry of mercury in the environment. Elsevier/North Holland Biomedical Press, Amsterdam, The Netherlands.

CRAIG, P. J., AND P. A. MORETON. 1985. The role of speciation in mercury methylation in sediments and water. Environ. Pollut. Ser. B 10: 141–158.

DONNAN, J. A. 1986. Mercury pollution in the Wabigoon–English River system: a socio-economic assessment of remedial measures. Ontario Ministry of the Environment. 80 p. (Available from Corporate Policy and Planning Branch, Ontario Ministry of the Environment, 135 St. Clair Ave. W., Toronto, Ont.)

FAGERSTROM, T., AND A. JERNELOV. 1971. Formation of methylmercury from aerobic organic sediment. Water Res. 5: 753–754.

FURUTANI, A., AND J. W. M. RUDD. 1980. Measurement of mercury methylation in lake water and sediment samples. Appl. Environ. Microbiol. 40: 770–776.

JACKSON, T. A. 1986. Methylmercury levels in a polluted prairie river–lake system: seasonal and site-specific variations and the dominant influence of trophic conditions. Can. J. Fish. Aquat. Sci. 43: 1873–1887.

1988. The mercury problem in recently formed reservoirs of northern Manitoba (Canada): effects of impoundment and other factors on the production of methylmercury by microorganisms in sediments. Can. J. Fish. Aquat. Sci. 45: 97–121.

JACKSON, T. A., J. W. PARKS, P. D. JONES, R. N. WOYCHUK, J. A. SUTTON, AND J. D. HOLLINGER. 1982. Dissolved and suspended mercury species in the Wabigoon River (Ontario, Canada); seasonal and regional variations. Hydrobiologia 92: 473–487.

JACKSON, T. A., AND R. N. WOYCHUK. 1980. The biogeochemistry and distribution of mercury in the Wabigoon River system. *In* T. A. Jackson [ed.] Mercury pollution in the Wabigoon/English River system of northwestern Ontario and possible remedial measures: a progress report. Published by Government of Canada (Dep. of Environment) and Government of Ontario (Ministry of the Environment). 28 p.

JENSEN, S., AND A. JERNELOV. 1969. Biological methylation of mercury in aquatic organisms. Nature (Lond.) 223: 753–754.

KUDO, A. 1977. Equilibrium concentrations of methylmercury in Ottawa River sediments. Nature (Lond.) 270: 419–420.

LANGLEY, D. G. 1973. Mercury methylation in an aquatic environment. J. Water Pollut. Control Fed. 45: 44–51.

LINDBERG, S. E., AND R. C. HARRIS. 1977. Release of mercury and organics from resuspended near-shore sediments. J. Water Pollut. Control Fed. 49: 2479–2487.

OLSON, B. H., AND R. C. COOPER. 1976. Comparison of aerobic and anaerobic methylation of mercuric chloride by San Francisco Bay sediments. Water Res. 10: 113–116.

ONTARIO MINISTRY OF THE ENVIRONMENT. 1981. Water quality data, Ontario lakes and streams, 1980. Water Resources Branch, Ontario Ministry of the Environment, Toronto, Ont. 670 p.

ONTARIO WATER RESOURCES COMMISSION. 1970. A water use study of the Wabigoon River. 38 p. (Available from Chief, Water Resources Assessment, Ontario Ministry of the Environment, Thunder Bay, Ont.)

OTTAWA RIVER PROJECT GROUP. 1979. Mercury in the Ottawa River. Environ. Res. 19: 231–243.

PARKS, J. W. 1976. Mercury in sediment and water in the Wabigoon–English River system, 1970–75. Ont. Minist. Environ. Tech. Rep. 28 p. (Available from Chief, Water Resources Assessment, Ontario Ministry of the Environment, Thunder Bay, Ont.)

PARKS, J. W., AND A. L. HAMILTON. 1987. Accelerating recovery of the mercury-contaminated Wabigoon/English River system. Hydrobiologia 149.

PARKS, J. W., J. D. HOLLINGER, AND P. M. ALMOST. 1980. The transport and dynamics of mercury in the Wabigoon River system. *In* T. A. Jackson [ed.] Mercury pollution in the Wabigoon–English River system of northwestern Ontario: a progress report. Published by Government of Canada (Dep. of Environment) and Government of Ontario (Ministry of the Environment).

PARKS, J. W., J. A. SUTTON, AND J. D. HOLLINGER. 1984. Mercury contamination of the Wabigoon–English Winnipeg River system — causes, effects and possible remedial measures, p. 1–352. *In* Mercury pollution in the Wabigoon/English River system of northwestern Ontario and possible remedial measures. Final Rep. of the Steering Committee. Vol. 1 and 2. Published by Government of Canada (Dep. of Environment) and Government of Ontario (Ministry of the Environment). 570 p.

PARKS, J. W., J. A. SUTTON, AND A. LUTZ. 1986. Effect of point and diffuse source loadings on mercury concentrations in the Wabigoon River: evidence of a seasonally varying sediment–water partition. Can. J. Fish. Aquat. Sci. 43: 1426–1444.

RAMLAL, P., J. W. M. RUDD, AND R. E. HECKY. 1986. Measurement of specific rates of mercury methylation and degradation and their use in determining factors controlling net rates of mercury methylation. Appl. Environ. Microbiol. 51: 110–114.

ROBINSON, J. B., AND O. H. TUOVINEN. 1984. Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biological and genetic analyses. Microbiol. Rev. 48: 95–124.

RUDD, J. W. M., AND M. A. TURNER. 1983. Inhibition of mercury and selenium bioaccumulation from water and surface sediments of large *in situ* enclosured by the addition of suspended sediments. Can. J. Fish. Aquat. Sci. 40: 2218–2227.

RUDD, J. W. M., M. A. TURNER, A. FURUTANI, A. SWICK, AND B. E. TOWNSEND. 1983. A synthesis of recent research with a view towards mercury amelioration. Can. J. Fish. Aquat. Sci. 40: 2206–2217.

SCHWOERBEL, J. 1987. Handbook of limnology. John Wiley & Sons, Toronto, Ont. p. 228.

SPANGLER, W. J., J. L. SPIGARELLI, J. M. ROSE, R. S. FLIPPIN, AND H. H. MILLER. 1973. Degradation of Methylmercury by bacteria isolated from environmental samples. Appl. Microbiol. 25: 488–493.

STUMM, W., AND J. J. MORGAN. 1981. Aquatic chemistry. John Wiley & Sons, Toronto, Ont. 780 p.

WATER SURVEY OF CANADA. 1983. Historical streamflow summary. Environment Canada, Ottawa, Ont. 520 p.

WOLLAST, R., G. BILLEN, AND F. T. MCKENZIE. 1978. Behaviour of mercury in natural systems and its global cycle, p. 145–166. *In* A. D. McIntyre and C. F. Mills [ed.] Ecological toxicology research: effects of heavy metal and organohalogen compounds. Plenum Press, New York, NY.

WRIGHT, D. R., AND R. D. HAMILTON. 1982. Release of methyl mercury from sediments: effects of mercury concentration, low temperature and nutrient addition. Can. J. Fish. Aquat. Sci. 39: 1459–1466.

Perturbation, Planktivory, and Pelagic Community Structure: The Consequence of Winterkill in a Small Lake[1]

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Hall, D. J., and T. J. Ehlinger. 1989. Perturbation, planktivory, and pelagic community structure: the consequence of winterkill in a small lake. Can. J. Fish. Aquat. Sci. 46: 2203–2209.

The top predator (largemouth bass, *Micropterus salmoides*) in eutrophic Wintergreen Lake, Michigan, was eliminated by successive winterkills in 1978 and 1979. Within 2 yr, the golden shiner (*Notemigonus crysoleucas*), a facultative planktivore, dominated the fish community. The zooplankton, previously consisting of large *Daphnia pulex* and *D. galeata mendotae*, concomitantly shifted to a community consisting of smaller species: *Bosmina* and small copepods. In situ fish enclosure experiments in 1981 and 1982 revealed that the large *Daphnia* species would grow in Wintergreen Lake in the absence of the golden shiner. Golden shiners exhibit both a particulate and filtering mode of planktivory on large and small zooplankton, respectively. This flexible feeding behavior suggests that golden shiners are able to prevent the reestablishment of *Daphnia*, while *Bosmina* and the other small zooplankton support a high and apparently stable golden shiner density.

Le prédateur au sommet de l'échelle (achigan à grande bouche, *Micropterus salmoides*) du lac eutrophe Wintergreen au Michigan, a été éliminé par des hécatombes hivernales successives en 1978 et en 1979. En deux ans, la chatte de l'Est, *Notemigonus crysoleucas*, un planctivore facultatif, est devenue l'espèce dominante chez les poissons. Le zooplancton, qui était constitué auparavant des gros organismes *Daphnia pulex* et *D. galeata mendotae*, s'est transformé durant la même période en une communauté qui était constituée d'espèces plus petites que les précédentes, soit *Bosmina* et de petits copépodes. Des essais sur place effectués en 1981 et en 1982 à l'aide d'enceintes afin d'exclure les poissons ont montré que les organismes de grande taille *Daphnia* se multiplieraient dans le lac Wintergreen en l'absence de la chatte de l'Est. Cette espèce planctivore se nourrit du gros plancton en l'avalant et du petit zooplancton par filtration. Ce mode souple d'alimentation donne à penser que la chatte de l'Est peut rendre impossible le rétablissement de *Daphnia* alors que *Bosmina* et d'autres petits organismes zooplanctoniques contribuent au maintien d'une densité élevée et apparemment stable de la chatte de l'Est.

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Unpredictable events such as species introductions, changes in water level, or winterkill can produce significant and lasting changes in lacustrine fish and zooplankton assemblages (Mahon and Balon 1977; Kitchell and Kitchell 1980; Tonn and Magnuson 1982; Rahel 1984; Tonn 1985). These unpredictable and often cataclysmic disturbances may alter strong predator–prey interactions between fish and zooplankton and trigger cascading changes in the ecological interactions among zooplankton and phytoplankton (Shapiro and Wright 1984; Carpenter et al. 1985; McQueen et al. 1986). Following such perturbations, these altered fish and plankton communities may not return to their original species and numerical configurations; instead very different community structures may persist.

In this study we examine the long-term consequences of winterkill of the top predator, largemouth bass (*Micropterus salmoides*), on the fish and zooplankton communities of a small lake. We attempt to document the causes of these community level changes by means of before and after winterkill comparisons, an in situ planktivore exclosure study, and laboratory fish–zooplankton foraging experiments.

The System

Wintergreen Lake, Kalamazoo County, Michigan, USA, is a small (15 ha), shallow (6.3 m maximum, 3.5 m mean depth), hardwater lake. Macrophytes cover nearly 40% of the lake's surface. Most of the nutrient influx comes from the droppings of thousands of migrant and resident waterfowl (Manny et al. 1975). Runoff from cultivated fields and an intermittent stream from a dairy farm feedlot also contribute nutrients. When the lake overflows, it drains 0.3 km west into Gull Lake which once served as a source of fish immigration; however, the overflow stream was incorporated into an underground drain in the 1960s, and fish migration is no longer possible. Wintergreen is a hypereutrophic lake (>1000 mg $C \cdot m^{-2} \cdot d^{-1}$ annual mean pelagic productivity (Wetzel 1983)), subject to numerous phytoplankton blooms and low Secchi disc transparencies (mean 1.3 m, range 0.4–3.7 m). The phytoplankton is dominated by *Chlamydomonas*, cryptomonads, and other small flagellated cells from before or just after ice-out in March until late May or June when large blue-green algae, especially *Anabaena, Aphanizomenon, or Microcystis*, begin to predominate. By August at least one of these large filamentous blue-green species is always dominant both in mass and numbers (Manny 1971; Duong 1972). Non-N_2-fixing phytoplankton rapidly reduce concentrations of combined nitrogen to limiting levels by early summer (Wetzel, 1983). From that time until September, N_2-fixing algae often predominate. It is unlikely that phytoplankton are phosphorus limited in Wintergreen Lake (R. G. Wetzel, Biology Department, University of Michigan, Ann Arbor, MI 48109).

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Winterkill: Before and After

The fish fauna of Wintergreen Lake was surveyed by G. P. Cooper in 1936 (Michigan Department of Conservation, Institute of Fisheries Research, unpubl. data), Fetterolf (1952), and W. C. Johnson in the 1960s and 1970s (Kellogg Biological Station, Hickory Corners, MI 49060, unpubl. data) (Table 1). These surveys revealed a typical warm-water, soft-bottom lake fish community, dominated by Centrarchidae with the largemouth bass as the top predator. Wintergreen Lake is subject to occasional partial winterkill (W. C. Johnson, pers. comm.), but prior to the winter of 1977, either individuals of each fish species population survived the periods of low dissolved oxygen or individuals were able to recolonize from nearby Gull Lake by swimming up the former outlet stream during high water levels. The winters of 1977 and 1978 were particularly severe in Michigan, with 4 mo of continuous ice and snow cover (W. C. Johnson, pers. comm.). Dissolved oxygen dropped to <1 $mg \cdot L^{-1}$ during these winters (Table 2). These conditions resulted in a massive fish winterkill. Over 70% of the 7000 dead fish that were found and removed at ice-out in 1977 were bluegills (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) (W. C. Johnson, pers. comm.). Subsequent gill net and seine surveys plus occasional angling from 1979 to date have failed to reveal any bluegills or largemouth bass in the lake. Yellow perch (*Perca flavescens*), pumpkinseed (*Lepomis gibbosus*), yellow bullhead (*Ictalurus natalis*), bowfin (*Amia calva*), and especially the golden shiner (*Notemigonous crysoleucas*) are currently the predominant fishes (see Table 1). Following the 1977–78 winterkills, the population of golden shiners has increased greatly and this species is now overwhelmingly dominant (Table 1).

Concurrent with this change in the fish community, the zooplankton species abundance pattern shifted from a community dominated by large cladocerans (*Daphnia pulicaria* and *D. galeata mendotae*, >1.3 mm body length) to a system composed primarily of small cladocerans (*Bosmina longirostris, Daphnia parvula, Ceriodaphnia reticulata, Chydorus sphericus*, and *Diaphanosoma brachyurum*, all <1.3 mm), the copepods (*Tropocyclops prasinus, Mesocyclops edax*, and *Diaptomus pallidus*), and in the spring the rotifers (primarily *Keratella* sp. and *Synchaeta* sp.) and large ciliates. Prior to the 1977–78, fish winterkill large *Daphnia* (1.3–2.5 mm) were found in abundance throughout all or much of the year, often exceeding $100 \cdot L^{-1}$ (Threlkeld (1979), Haney and Hall (1975), and in the

TABLE 1. Fish species abundances in Wintergreen estimated by various methods in different years. The 1952 abundances were estimated using the Schnabel mark–recapture method (Fetterolf 1952). The 1963 and 1981 relative abundances were estimated from overnight gill net catches scaled to ratio counts. The 1977 dead fish counts represent all dead fish encountered along shore at ice-out. The 1987 data are based on Schnabel multiple mark–recapture methods (M. Johannes, Biology Department, York University, Toronto, Ontario M3J 1P3, pers. comm.).

	1952 (mark–recapture)	1963 (relative catch per effort)	1977 (dead fish count)	1981 (relative catch per effort)	1987 (mark–recapture)
Amiidae					
Amia calva bowfin)	46 (19–155)[a]	1	69	1	1
Centrarchidae					
Lepommis gibbosus (pumpkinseed)	3314 (1974–6025)	5	135	10	6967 (4767–10 655)
L. macrochirus (bluegill)	12 149 (9136–16 550)	10	4314	0	0
Micropterus salmoides (largemouth bass)	2654 (2420–2818)	5	519	0	0[c]
Cyprinidae					
Notemigonus crysoleucas (golden shiner)	—[b]	1	0	70	422 138[d] (368 055–489 587)
Ictaluridae					
Ictalurus natalis (yellow bullhead)	166 (127–240)	1	119	3	
Percidae					
Perca flavescens (yellow perch)	2276 (1198–5122)	10	1800	30	15 325 (6538–36 143)

[a] 95% confidence limits.

[b] Present but not captured in trap nets.

[c] Excluding stocked cohort (see text).

[d] YOY not included.

TABLE 2. Dissolved oxygen (mg·L^{-1}) in Wintergreen Lake during the winters of 1977 and 1978 (M. Klug, Kellogg Biological Station, Hickory Corners, MI 49060). Underlined values of dissolved oxygen are lethal to largemouth bass and bluegill. N.D. = not determined.

Date (mo-d-yr)	Surface	1 m	2 m	3 m	4 m	5 m	6 m
1-17-77	0.43	0.62	$\underline{0.49}$	0.61	$\underline{0.50}$	$\underline{0.48}$	0.52
2-1-77	0.72	0.72	$\underline{0.24}$	0.86	0.62	0.60	0.60
2-14-77	$\underline{0.36}$	1.16	$\underline{0.20}$	0.56	$\underline{0.40}$	$\underline{0}$	$\underline{0}$
2-28-77	0.84	$\underline{0.30}$	$\underline{0.08}$	$\underline{0.12}$	$\underline{0}$	$\underline{0}$	$\underline{0}$
3-17-77	6.64	6.34	6.48	6.08	6.12	5.66	6.14
3-29-77	12.44	13.82	13.62	12.44	12.60	12.60	12.26
1-31-78	N.D.	2.58	2.64	1.67	0.95	$\underline{0.18}$	$\underline{0.17}$
2-13-78	1.01	1.56	1.52	$\underline{0.46}$	$\underline{0.34}$	$\underline{0.24}$	$\underline{0.24}$
2p-27-78	$\underline{0.45}$	$\underline{0.38}$	0.58	$\underline{0.14}$	$\underline{0.15}$	$\underline{0.22}$	$\underline{0.26}$
3-13-78	N.D.	$\underline{0.12}$	$\underline{0.09}$	$\underline{0.12}$	$\underline{0}$	$\underline{0}$	$\underline{0.29}$
3-27-78	N.D.	2.64	0.83	$\underline{0.30}$	$\underline{0.05}$	$\underline{0.08}$	$\underline{0.20}$
4-11-78	19.43	19.04	18.87	18.29	13.69	2.26	$\underline{0.22}$

1960s by D. C. McNaught (Ecology Department, University of Minnesota, Minneapolis, MN 55455)). *Ceriodaphnia* were always less abundant than *Daphnia*, usually by a factor of 4 times, while *Bosmina* and the calanoid copepod *Diaptomus* were always rare ($<1 \cdot L^{-1}$). A single vertical plankton tow net sample taken in August 1978 revealed the presence of both *D. pulicaria* and *D. galeata mendotae* (R. Wetzel, pers. comm.), but in July 1980, no large *Daphnia* were found in two vertical tow net samples (C. E. Goulden, Division of Limnology and Ecology, 19th and The Parkway, Philadelphia, PA 19103). Our more extensive horizontal and vertical sampling with plankton nets in 1981 and 1982 (see below) contained no large-bodied *Daphnia* spp. *Bosmina* and *Diaptomus*, however, became abundant in 1978 and have remained abundant in the community with densities often exceeding $100 \cdot L^{-1}$.

The coincidence of the increase in golden shiner abundance with the demise of the large daphnid species following the winterkill in Wintergreen Lake suggested intense planktivory as the mechanism generating the shift in size and species structure of the zooplankton community. A test of this predation hypothesis involved a field experiment designed to evaluate directly the success of large *Daphnia* spp. in Wintergreen Lake in the absence of the golden shiner. Alternative explanations of the demise of the large daphnids include (1) no recolonization source of *Daphnia*, (2) inappropriate food for *Daphnia*, (3) competitive exclusion, and (4) invertebrate predation. Evidence from our field experiment and other observations allows us to reject these alternative hypotheses.

Methods

A single fish enclosure was constructed, measuring 1 × 1 × 4.3 m deep; it consisted of a floating square wood and styrofoam frame from which a 0.2-mm-mesh rayon net extended to the lake bottom. Two wire stays prevented this fabric column from collapsing. The bottom was closed off with a 1 × 1 m piece of 1.3-cm-mesh hardware cloth that rested in the sediments. To test the retention of zooplankton inside this column, an aquarium was divided in half using a piece of rayon net, and zooplankters from Wintergreen Lake of sizes 0.2–2 mm body length were introduced on one side. After 2 h, no individuals were found in the other half of the aquarium.

The ability of large daphid species to survive and reproduce in Wintergreen Lake in the absence of fish predation was tested directly by introducing small populations of *D. pulicaria* and *D. galeata mendotae* into the fish exclosure placed in the lake. Our intent was to determine if these populations of large *Daphnia* would persist without golden shiner predation. The fish exclosure was placed in the lake and inoculated in both 1981 and 1982 with several thousand *D. galeata mendotae* ($<2 \cdot L^{-1}$, initial density) and a few hundred *D. pulicaria* collected from a nearby eutrophic lake.

Duplicate vertical samples of zooplankton were taken with a 15-cm-diameter, 156-μm-mesh plankton net. Collections were made at midday every 4–9 d from July 20 to August 6, 1981, and from May 7 to August 11, 1982, from bottom to surface both inside and outside the exclosure. Samples were preserved in 95% ethanol. Duplicate aliquots were removed from each sample and all crustacean zooplankton identified. Generally, at least 30 individuals of each species were enumerated per aliquot. Total body length and brood size were also determined. Rotifers were treated only in a qualitative manner, since a 156-μm or larger mesh net was used to collect these very small (150–300 μm) plankton. Additional 100-m-long oblique tows were taken every month with a 0.5-m-diameter, 500-μm-mesh net to check for the presence of large *Daphnia* in the lake.

Fish abundances were estimated by various methods depending on the goals of the different studies performed on Wintergreen Lake. Fetterolf (1952) performed a Schnabel (1938) mark–recapture method of estimating fish abundance. Fish were captured by hoop nets, a Great Lakes trap net, and by angling. Small fish (<120 mm SL) were not included, apparently because of the large (3.75–6.25 cm) mesh size of the nets. The number of recaptured marked fish ranged from 654 bluegills down to 94 yellow bullheads. In 1963 (W. C. Johnson, pers. comm.) and in 1981, a total of 24 overnight experimental gill net sets provided a catch per unit effort and thus a relative abundance estimate. Fish were periodically angled throughout the 1960s and early 1970s. In late summer 1987, M. Johannes (Biology Department York University, York, Ont., pers. comm.) estimated the fish abundances using Schnabel methods from multiple census mark–recapture data (Schnabel 1938; Ricker 1975). Fish were marked and recaptured during at least six successive intervals using a 31 × 9.8 m, 1-mm-mesh purse seine and a 31 × 1.8 m, 5-mm beach sieve. Each census interval was completed when 5 beach seining and 10 purse seining sites spread around the entire lake had been randomly fished. The number of recaptures ranged from 7700 golden shiners to 780 pumpkinseeds.

Fish were collected for gut analysis using gill nets on three midsummer dates during the summer of 1981 and again in 1986. Fish were removed 1 h after set, iced, and later frozen. Stomachs were removed and injested prey identified, measured, and counted in the laboratory.

Results and Discussion

Fish Predation Hypothesis

The population of *D. galeata mendotae* grew rapidly after its introduction to the fish exclosure both in 1981 and 1982 (Fig. 1A). We report in detail only the results of 1982 because of the longer duration of the observation (the results from 1981 show similar patterns). By May 26, 1982, the population had reached a density of $>100 \cdot L^{-1}$ (Fig. 1A). On May 28 we released

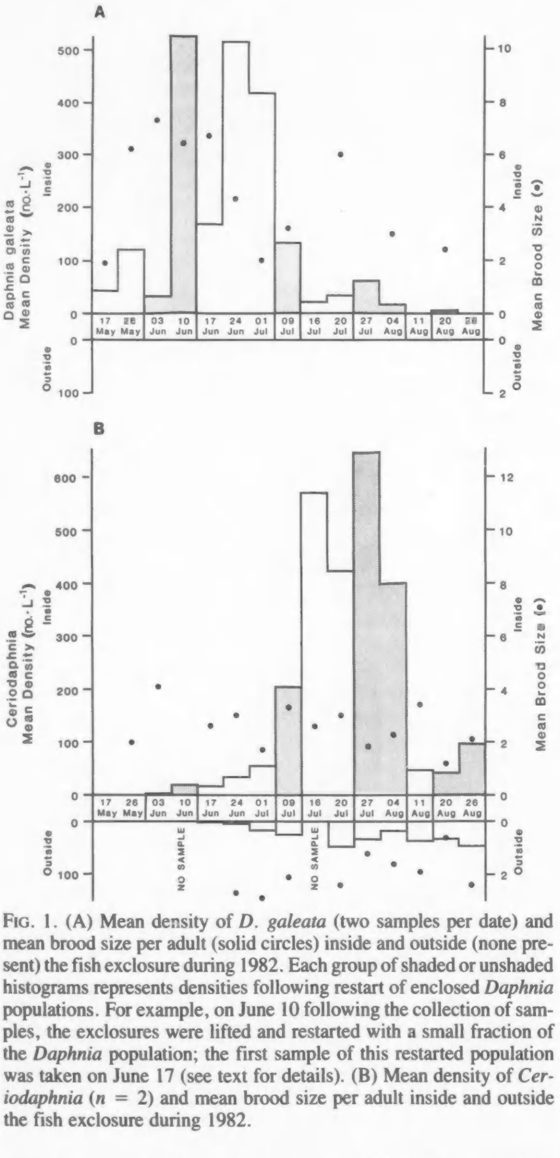

FIG. 1. (A) Mean density of *D. galeata* (two samples per date) and mean brood size per adult (solid circles) inside and outside (none present) the fish exclosure during 1982. Each group of shaded or unshaded histograms represents densities following restart of enclosed *Daphnia* populations. For example, on June 10 following the collection of samples, the exclosures were lifted and restarted with a small fraction of the *Daphnia* population; the first sample of this restarted population was taken on June 17 (see text for details). (B) Mean density of *Ceriodaphnia* (n = 2) and mean brood size per adult inside and outside the fish exclosure during 1982.

nearly all of these *Daphnia* (about 10^6 individuals) into Wintergreen Lake by simply lifting the exclosure from the lake and resuspending it into a new column of lake water. We retained about 10^3 *Daphnia* in order to reseed the fish exclosure. This procedure provided three important changes. (1) The rapidly growing *Daphnia* population was culled back to a much smaller size, thereby reducing the possibility of a crash due to overcrowding or food limitation. For example, in May, the Secchi disc water transparency remained at 1.0 m in the open lake whereas in the fish exclosure the transparency increased from 1.0 to 4 m as the *Daphnia* density increased, suggesting intense grazing on the algae. (2) A new column of water was enclosed, thereby providing the enclosed *Daphnia* population with ambient lake conditions (phytoplankton, etc.). (3) The *Daphnia* released into the open lake served as an inoculum that could persist and grow if lake conditions permitted. The *Daphnia* population and other zooplankton were culled back (and the lake "seeded") six times in 1982 (May 28, June 10, July1, 9, and 20, and August 4). In 1981, the exclosure was never restarted as above.

TABLE 3. Secchi disc transparencies (m) in Wintergreen Lake. There was no significant difference between 1982 and 1971–72 at the 5% level using the Mann-Whitney one-tailed test.

Date		1982	1971–72
May	1		0.9
	6	1.0	
	15		2.2
	19	1.0	
	28	0.9	1.7
	30		
June	3	1.8	
	13		1.1
	17	3.0	
	22	1.6	
	27	1.5	
July	1	1.0	1.5
	6		1.2
	9	1.7	
	13	1.0	
	16	1.8	
	20		1.2
	24		1.3
August	3		0.7
	4	1.2	
	10		1.2
	24		1.7
	26	1.2	

In early summer each "reinitiated" *D. galeata mendotae* population grew rapidly inside the exclosure. These population surges resulted from a combination of high birth rate (mean brood size per adult of 5.5) and high survival. The population did not grow as fast in July, and by August 11 the population declined and went extinct even though brood size was greater than two eggs per adult. *Daphnia pulicaria*, although never achieving large population sizes, persisted until August 4.

Although we used only one fish exclosure, the six times that we reinitiated the enclosed *Daphnia* population (Fig. 1) permitted us to test repeatedly the golden shiner planktivory hypothesis over those months when fish feed most actively and when the major algal succession occurs. Also, the 1981 results substantiate those of 1982. We did not introduce fish into cages, since confining fish to a fixed 4.3-m^3 cage would probably result in unrealistically high predation rates and thus strongly bias in favor of the fish predation hypothesis.

Alternative Hypotheses

In addition to the fish predation hypothesis, there are several other plausible explanations why the large *Daphnia* spp. have not recolonized Wintergreen Lake. Evidence collected from the exclosure experiments and lake samples allows us to reject these alternate hypotheses.

(1) *Daphnia* have not been reintroduced to Wintergreen Lake. This hypothesis is easily dismissed. Even though we intentionally released 10^6 *Daphnia* into Wintergreen Lake from the fish exclosure, no individuals of the large *Daphnia* spp. were ever

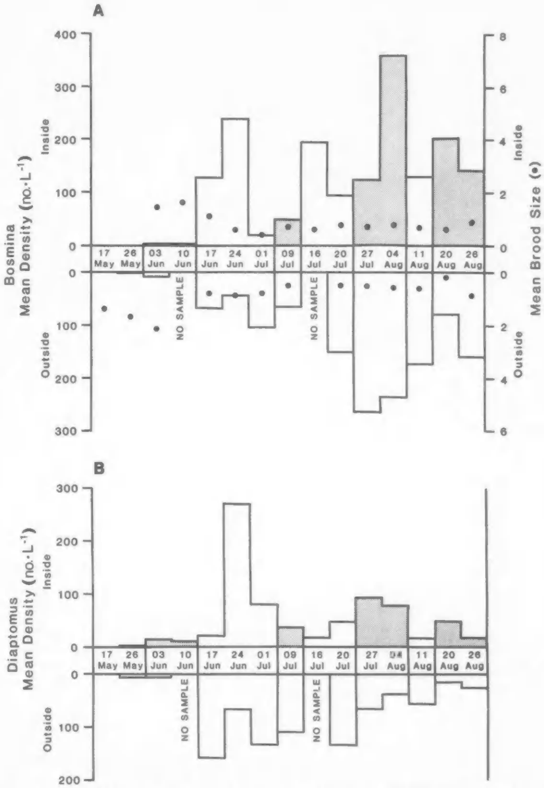

FIG. 2. (A) Mean density of *Bosmina* ($n = 2$) and mean brood size (solid circles) inside and outside the fish exclosure during 1982. (B) Mean density of *D. pallidus* ($n = 2$) inside and outside the fish exclosure during 1982.

found in lake samples. Daphnid resting eggs (ephippia) occur in the sediments of Wintergreen Lake in densities often exceeding $1000 \cdot m^{-2}$ (R. G. Wetzel, unpubl. data). These ephippia were produced by the prewinterkill *Daphnia* population and perhaps supplemented by ephippia transported from other lakes on the feathers or in the guts of migrating waterfowl. Sediments collected from Wintergreen Lake in 1983 were placed in aerated containers in the laboratory. *Daphnia galeata mendotae* appeared in 3 of the 20 containers. Therefore some of these ephippia were viable and potentially could reinitiate a *Daphnia* population to the lake.

(2) *Daphnia* have been unable to grow in Wintergreen Lake because of insufficient and/or deleterious food conditions following the winterkill. This hypothesis is untenable, since *Daphnia* grew rapidly in the fish exclosures containing the natural complement and (initially) the natural densities of lake phytoplankton and other seston. Recall that the *Daphnia* population continued to grow following each resuspension of the exclosure from May until August. However, this hypothesis is compatible with the disappearance of *Daphnia* from the exclosure in August. Threlkeld (1979) observed the same seasonal disappearance of *Daphnia* from Wintergreen Lake previous to winterkill. We discuss the implications of seasonally changing resources below, but the fact that large *Daphnia* spp. have not been collected from Wintergreen Lake at any time of year following the winterkill of 1978 suggests the operation of a more pervasive process.

We detected no change in the total biomass of the phytoplankton community after the winterkill and the elimination of *Daphnia*. Pre- and post-winterkill comparisons of Secchi disc depths showed no differences ($P >> 0.05$, Mann-Whitney *U*-test; Table 3). Furthermore, the median *Daphnia* brood size in the enclosures (Fig. 1A) was twice the size measured in prewinterkill in Wintergreen Lake by Threlkeld (1979), suggesting that food conditions, if anything, have improved.

(3) Other zooplankton species competitively excluded large *Daphnia* spp. from Wintergreen Lake. *Bosmina*, *Ceriodaphnia*, and *Diaptomus* are purported to consume similar resources and potentially compete with *Daphnia* (Lynch 1978; DeMott and Kerfoot 1982; Goulden and Hornig 1980; Richman et al. 1980). The small cladoceran *Bosmina* (<1.0 mm) and calanoid copepod *Diaptomus pallidus* (~1.5 mm adult length) are the most abundant herbivorous zooplankters in Wintergreen Lake. Their respective seasonal abundances inside and outside the exclosure were similar (Fig. 2A and 2B). If we utilize the lake abundance pattern as a benchmark, then neither species showed a response to the high density achieved by *Daphnia* in the fish exclosure. The seasonal pattern of *Bosmina* brood sizes was the same inside and outside the exclosure. By contrast, *Ceriodaphnia* (<1.0 mm) was never abundant in Wintergreen Lake (maximum density of $50 \cdot L^{-1}$), but the population inside the fish exclosure showed dramatic growth ($>500 \cdot L^{-1}$) during July (Fig. 1B). Maximal *Ceriodaphnia* densities coincided with minimal *Daphnia* densities. *Ceriodaphnia* densities in the exclosure then declined to $<100 \cdot L^{-1}$ in August. It is difficult to ascertain whether *D. parvula* (a smaller daphnid) had any effect on *D. galeata mendotae* inside the exclosure. Compared with ambient densities outside the exclosure, *D. parvula* was less abundant in 1982 but equally abundant in 1981 during the times that *D. galeata mendotae* populations were thriving inside the exclosure.

We conclude from these comparisons of species population dynamics inside and outside the fish exclosure that *D. galeata mendotae* is not excluded from Wintergreen Lake due to competition; certainly we found no evidence prior to July compatible with a competitive exclusion hypothesis because *D. galeata mendotae* was able to increase in the presence of the other species. During July and August, *Ceriodaphnia* may have competed for resources with large *Daphnia* in the fish exclosure. Consideration of brood sizes, egg development, and reproductive maturation rates (Hall et al. 1970) indicates that the *Ceriodaphnia* population in the enclosure achieved maximal growth potential during this period of summer. The enclosed *Daphnia* populations, in spite of relatively large brood sizes (from two to six eggs per adult) achieved, at best, barely positive growth rates in late July and August. Neill (1975) has demonstrated that *Ceriodaphnia* can competitively exclude young *D. magna* by driving resource levels below the maintenance demands of juvenile *Daphnia*. However, the continuing absence of large *Daphnia* species from Wintergreen Lake in the 1980s, especially during spring and early summer when *Daphnia* formerly reached densities of $>100 \cdot L^{-1}$ (Threlkeld 1979) and the disappearance of the 10^6 *Daphnia* released into the lake during June when *Ceriodaphnia* densities were $<3 \cdot L^{-1}$, cannot be accounted for by competitive exclusion. Furthermore, the enclosed *D. galeata mendotae* population exploded during May and June in the presence of their putative competitors, including rotifers which were abundant in the lake only in April and May.

(4) Invertebrate predation may prevent the establishment and/or persistence of *Daphnia*. There are three planktonic, invertebrate predators in Wintergreen Lake: the copepods (*T. prasinus* and *M. edax*) and the planktonic midge larvae (*Chaoborus punctipennis*). *Tropocyclops* is a very small predatory copepod (adult length 0.5 mm) which cannot prey on *Daphnia*. *Mesocyclops* (adult length 1.3 mm) can successfully prey upon the neonates of large *Daphnia* spp. Several investigators, however, have discovered that *Mesocyclops* normally selects much smaller prey including rotifers and *Bosmina* (Williamson and Gilbert 1980; Jamieson 1980). Apparently, *Mesocyclops* did not prey extensively on newborn *Daphnia*, since *Daphnia* increased rapidly in the fish exclosure even though *Mesocyclops* occurred above natural densities there (D. J. Hall, unpubl. data).

Chaoborus punctipennis grows through four larval instars reaching a maximum length of >12 mm. The 3rd and especially 4th instar *Chaoborus* can prey on juvenile *Daphnia* (cf. Pastorok 1980; Fedorenko 1975) and if very abundant might be an important predator. The following evidence is strongly suggestive, however, that *Chaoborus* is not responsible for the absence of *Daphnia* in Wintergreen Lake. (i) Inside and outside exclosure densities of *Chaoborus* were not different. *Chaoborus punctipennis* migrates into the sediments of Wintergreen Lake by day (R. G. Wetzel, Michigan State University, Hickory Corners, MI 49060, unpubl. data). It thus was free to reenter the exclosure through the large (1.3-cm) mesh bottom screen at night. *Chaoborus* densities were estimated at midnight in early July 1981 from duplicate samples taken inside the fish exclosure with a 3-L van Dorn water bottle and outside the exclosure with a 15-cm-diameter plankton net towed for 38 m at a depth from 1 to 3 m. The inside densities were 3.0 and 3.1 *Chaobrous*$\cdot L^{-1}$; the outside densities were 3.0 and 7.0$\cdot L^{-1}$. Unfortunately, no other comparisons were possible, since all other zooplankton collections were made at midday when *Chaoborus* was not found in the water column. (ii) Prewinterkill densities were equal to or higher than postwinterkill estimates. *Chaoborus* densities previous to winterkill were 2.6 and 3.0$\cdot$L^{-1} in replicated nighttime Clarke-Bumpus net tows taken on August 15, 1967 (D. McNaught, pers. comm.), and 5.32 ± 0.84$\cdot$L^{-1} from a series of midnight vertical net tows taken in June–July 1976 by Threlkeld (1979). Postwinterkill densities ranged from 0.7 to 10.5$\cdot$L^{-1} extrapolated from counts of *Chaoborus* in daytime Eckman sediment grabs taken in 1981 (R. G. Wetzel, unpubl. data) and a mean density of 0.40 ± 0.1$\cdot$L^{-1} from nine vertical tows taken at midnight in August 1987. (iii) The *C. punctipennis* population in Wintergreen Lake, at least in spring (April–May), consists entirely of 1st and 2nd instars which are incapable of catching or killing *Daphnia*. Recall that this is the season when the *Daphnia* population was introduced and grew rapidly inside (but not outside) the fish exclosure. (iv) Experimental field studies indicate that large *Daphnia* spp. usually persist in the presence of elevated *Chaoborus* densities, especially when smaller "preferred" prey such as *Bosmina*, rotifers, and copepods are abundant (Neill 1981; Giguere 1979).

Species Composition and Relative Abundance of the Fish Community

The golden shiner was first recorded from Wintergreen Lake by G. P. Cooper in 1936 (unpubl. data). In the next fish survey of the lake by Fetterolf (1952), the golden shiner, although present, was not quantified probably because this species was not caught by angling or in the coarse-mesh (3.75–6.25 cm) trap nets used in this study. W. C. Johnson (unpubl. data) confirmed the presence of golden shiners from experimental gill net sets in 1963. However, the golden shiner was less abundant than any centrarchid species (Table 1). W. C. Johnson (unpubl. data) confirmed the presence of bass and bluegills through 1975 from his annual angling in the lake. (No other anglers were allowed access to Wintergreen Lake.) Following the winterkill of 1977, W. C. Johnson (unpubl. data) found 519 dead bass and 4314 dead bluegills at ice-out in late March, but no dead golden shiners. Since 1977 no bass or bluegills have been recorded from Wintergreen Lake in spite of intensive sampling efforts. By contrast, the golden shiner (>30 mm SL) in 1987 had reached a total population size of 273 699 with a 95% confidence interval of 219 000 – 340 000 as estimated by M. Johannes (unpubl. data) using multiple census–mark–recapture methods (Schnabel 1938; Ricker 1975). Although we have no absolute population estimates of golden shiners prior to winterkill, the *relative* abundances estimates suggest that the golden shiner was probably never as abundant as the bluegill (population size estimate 12 149 in 1952, Table 1).

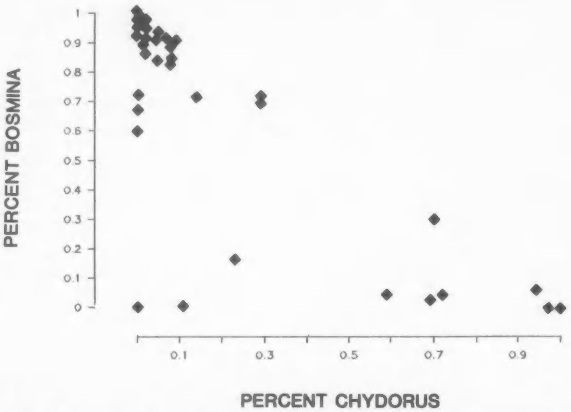

FIG. 3. Representative diet profile of the golden shiner (n = 33) in 1981. Dominant numerical prey were *Bosmina* and *Chydorus* with miscellaneous small littoral invertebrates constituting the remainder.

The golden shiner population appears to have increased by at least one (possibly two) orders of magnitude following winterkill and is now the dominant fish in Wintergreen Lake. The yellow perch has also increased to a population of 15 325 individuals. The pumpkinseed is the only centrarchid to survive the winterkill, and its abundance in 1987 had reached 2833 individuals comparable with the 1952 estimate of 3314 individuals (Table 1).

The fish predation hypothesis most likely explains the elimination of large *Daphnia* from Wintergreen Lake. This hypothesis receives further support from consideration of the golden shiner density, feeding behavior, and alternative prey availability in Wintergreen Lake.

The density of golden shiners is at least 1.0 fish$\cdot$m^{-3} as estimated by dividing the population estimates in Table 1 by the inhabitable (summer oxygenated) volume of the lake (Wetzel 1983, fig. 3–11). The golden shiner is a size-selective planktivore that prefers large zooplankton over small forms, but can filter feed on abundant small zooplankton such as *Bosmina* (Ehlinger 1989). These observations suggest that the golden shiners are sufficiently abundant in Wintergreen Lake to encounter and eat any *Daphnia* that hatch out from resting eggs and that the large golden shiner population is being maintained by foraging on small zooplankton, primarily *Bosmina, Chydorus*, and some littoral zone prey (Fig. 3).

Conclusions

All the largemouth bass and bluegill disappeared from Wintergreen Lake following two successive winterkills in 1977 and 1978. These two centrarchid species cannot survive the low dissolved oxygen concentrations of $<0.5\ \mathrm{mg \cdot L^{-1}}$ that occurred during these winters (Table 2) whereas the other species are more tolerant of low oxygen levels (Cooper and Washburn 1946; Moore 1942; Petrosky and Magnuson 1973; Schneberger 1970). The golden shiner became the dominant fish and large *Daphnia* were replaced by small-bodied zooplankton. We contend that the removal of the top-down control by the piscivorous bass (Carpenter et al. 1985; McQueen et al. 1986) led to the golden shiner population explosion which in turn led to the demise of large *Daphnia*. This reconfiguration of the fish and zooplankton communities has remained largely unchanged for 6 yr from 1981 to 1987.

If piscivorous fish are restocked in Wintergreen Lake, the current fish–zooplankton assemblage will probably revert to its prewinterkill structure (Carpenter et al. 1985). We are currently exploring the dynamics of community change by intentionally introducing a cohort of 700 young-of-the-year largemouth bass and tracking the long-term fish, zooplankton, and phytoplankton responses.

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References

Carpenter, S. R., J. F. Kitchell, and J. R. Hodgson. 1985. Cascading trophic interactions and lake productivity. BioScience 35: 634–639.

Cooper, G. P., and G. N. Washburn. 1946. Relation of dissolved oxygen to winter mortality of fish in Michigan lakes. Trans. Am. Fish. Soc. 76: 23–33.

DeMott, W. R., and W. C. Kerfoot. 1982. Competition among cladocerans: nature of the interaction between *Bosmina* and *Daphnia*. Ecology 63: 1949–1966.

Duong, T. P. 1972. Nitrogen fixation and productivity in a eutrophic hardwater lake: *in situ* and laboratory studies. Thesis, Michigan State University, East Lansing, MI. 241 p.

Ehlinger, T. J. 1989. Foraging mode switches in the golden shiner (*Notemigonus crysoleucas*). Can. J. Fish. Aquat. Sci. 46: 1250–1254.

Fedorenko, A. Y. 1975. Instar and species-specific diets in two species of *Chaoborus*. Limnol. Oceanogr. 20: 238–249.

Fetterolf, C. M. Jr. 1952. A population study of the fishes of Wintergreen Lake, Kalamazoo County, Michigan: with notes on movement and effect of netting on condition. Thesis, Michigan State University, East Lansing, MI. 127 p.

Giguere, L. 1979. An experimental test of Dodson's hypothesis that *Ambystoma* (a salamander) and *Chaoborus* (a phantom midge) have complementary feeding niches. Can. J. Zool. 57: 1091–1097.

Goulden, C. E., and L. L. Hornig. 1980. Population oscillations and energy reserves in planktonic Cladocera and their consequences to competition. Proc. Natl. Acad. Sci. USA 77: 1716–1720.

Hall, D. J., W. E. Cooper, and E. E. Werner. 1970. An experimental approach to the production dynamics and structure of freshwater animal communities. Limnol. Oceanogr. 15: 839–928.

Haney, J. F., and D. J. Hall. 1975. Diel vertical migration and filter-feeding activities of *Daphnia*. Arch. Hydrobiol. 75(4): 413–441.

Jamieson, C. D. 1980. The predatory feeding of copepodid stages III to adult *Mesocyclops leuckarti* (Claus), p. 518–537. *In* W. C. Kerfoot [ed.] Evolution and ecology of zooplankton communities. University Press of New England, Hanover, NH.

Kitchell, J., and J. F. Kitchell. 1980. Size-selective predation, light transmission, and oxygen stratification: evidence from the recent sediments of manipulated lakes. Limnol. Oceanogr. 25: 389–402.

Lynch, M. 1978. Complex interactions between natural coexploiters — *Daphnia* and *Ceriodaphnia*. Ecology 59: 552–564.

Mahon, R., and E. K. Balon. 1977. Fish community structure in lakeshore lagoons on Long Point, Lake Erie, Canada. Environ. Biol. Fishes 2: 71–82.

Manny, B. A. 1971. Interactions of dissolved and particulate nitrogen in lake metabolism. Thesis, Michigan State University, East Lansing, MI. 189 p.

Manny, B. A., R. G. Wetzel, and W. C. Johnson. 1975. Annual contribution of carbon, nitrogen, and phosphorus by migrant Canada geese to a hardwater lake. Verh. Int. Ver. Theor. Angew. Limnol. 19: 949–951.

McQueen, D. J., J. R. Post, and E. L. Mills. 1986. Trophic relationships in freshwater pelagic ecosystems. Can. J. Fish. Aquat. Sci. 43: 1571–1581.

Moore, W. G. 1942. Field studies on the oxygen requirements of certain freshwater fishes. Ecology 23: 319–329.

Neill, W. 1975. Experimental studies of microcrustacean competition, community composition and efficiency of resource utilization. Ecology 56: 809–826.

Neill, W. E. 1981. Impact of *Chaoborus* predation upon the structure and dynamics of a crustacean zooplankton community. Oecologia 48: 164–177.

Pastorok, R. A. 1980. Selection of prey by *Chaoborus* larvae: a review and new evidence for behavioral flexibility, p. 538–554. *In* W. C. Kerfoot [ed.] Evolution and ecology of zooplankton communities. University Press of New England, Hanover, NH.

Petrosky, B. R., and J. J. Magnuson. 1973. Behavioral responses of northern pike, yellow perch and bluegill to oxygen concentrations under simulated winterkill conditions. Copeia 1973: 124–133.

Rahel, F. J. 1984. Factors structuring fish assemblages along a bog lake successional gradient. Ecology 65: 1276–1289.

Richman, S., S. A. Bohon, and S. E. Robbins. 1980. Grazing interactions among freshwater calanoid copepods, p. 219–233. *In* W. C. Kerfoot [ed.] Evolution and ecology of zooplankton communities. University Press of New England, Hanover, NH.

Ricker, W. E. 1975. Computation and interpretation of biological statistics of fish populations. Bull. Fish. Res. Board Can. 191.

Schnabel, Z. E. 1938. Estimation of the total fish population in a lake. Am. Math. Mon. 45(6): 348–352.

Schneberger, E. 1970. A symposium on the management of midwestern winterkill lakes. 32nd Midwest fish and Wildlife Conference, Winnipeg, Manitoba, Canada. American Fisheries Society, Bethesda, MD.

Shapiro, J., and D. I. Wright. 1984. Lake restoration by biomanipulation: Round Lake, Minnesota, the first two years. Freshwater Biol. 14: 371–383.

Threlkeld, S. T. 1979. The midsummer dynamics of two *Daphnia* species in Wintergreen Lake, Michigan. Ecology 60: 165–179.

Tonn, W. M. 1985. Density compensation in *Umbra-Perca* fish assemblages of northern Wisconsin lakes. Ecology 66: 415–429.

Tonn, W. M., and J. J. Magnuson. 1982. Patterns in the species composition and richness of fish assemblages in northern Wisconsin lakes. Ecology 63: 1149–1166.

Wetzel, R. G. 1983. Limnology. 2nd ed. Saunders College Publishing, Philadelphia, PA. 767 p.

Williamson, C. E., and J. J. Gilbert. 1980. Variation among zooplankton predators: the potential of *Asplanchna, Mesocyclops*, and *Cyclops* to attack, capture, and eat various rotifer prey, p. 509–517. *In* W. C. Kerfoot [ed.] Evolution and ecology of zooplankton communities. University Press of New England, Hanover, NH.

Effect of Sea-Age on the Reproductive Potential of Atlantic Salmon (*Salmo salar*) in Eastern Canada

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Randall, R. G. 1989. Effect of sea-age on the reproductive potential of Atlantic salmon (*Salmo salar*) in eastern Canada. Can. J. Fish. Aquat. Sci. 46: 2210–2218.

Reproductive potential of Atlantic salmon (*Salmo salar*), defined as average eggs per fish in the spawning run, varied three-fold both within stocks over time and among stocks from different geographic areas. Eggs per spawner is a function of fecundity, sea-age, proportion of females, and female size; the latter three traits varied significantly among years for salmon in both the Miramichi and Restigouche Rivers, New Brunswick. Because all of the above traits are related to sea-age at maturity, eggs per spawner was significantly correlated with mean sea-age in both rivers (R^2 = 0.88 and 0.61, respectively). Among 10 different populations in eastern Canada, reproductive potential was also correlated with sea-age; for mean sea-ages (MSA) ranging from 1.0 to 1.8 yr, reproductive potential (RP) was defined by the power regression: $RP = 1831.26\ MSA^{1.30}$ ($R^2 = 0.64$ $P < 0.05$). Thus reproductive potential can be estimated for any population for which the sea-age composition of spawners is known. Assuming a target egg deposition rate of 2.4×10^4 eggs per hectare, required spawners varied inversely with reproductive potential among the 10 populations, from five spawners (MSA = 1.75 yr) to 16 spawners (MSA = 1.15 yr) per hectare.

Le potentiel de reproduction du saumon de l'Atlantique (*Salmo salar*), défini selon le nombre moyen d'oeufs par poisson qui participe à une montaison, a varié de trois ordres de grandeur à l'intérieur de mêmes stocks en fonction du temps, et entre stocks provenant de différents secteurs géographiques. Le nombre d'oeufs par reproducteur est une fonction de la fécondité, de l'âge en mer, de la proportion de femelles et de la taille de ces dernières; les trois dernières caractéristiques mentionnées ont varié de manière significative d'une année à l'autre dans les populations de saumon de la Miramichi et de la Restigouche au Nouveau-Brunswick. Étant donné que tous les traits susmentionnés se rapportent à l'âge en mer à la maturité, le nombre d'oeufs par reproducteur marque une corrélation significative avec l'âge moyen en mer dans les populations des deux rivières (R^2 = 0,88 et 0, 61 respectivement). Dans dix populations différentes de l'est du Canada, le potentiel de reproduction est également en corrélation avec l'âge en mer; pour un âge moyen en mer moyen (AMM) de 1,0 à 1,8 an, le potentiel de reproduction (PR) se trouve défini par la régression de puissance suivante : $PR = 1831{,}26\ AMM^{1{,}30}$ (R^2 = 0,64 P<0,05). C'est ainsi que le potentiel de reproduction peut être évalué pour toute population dont la composition par âge en mer des reproducteurs est connue. Avec un taux de dépôt des oeufs de $2{,}4 \times 10^4$ oeufs par ha pour objectif, le nombre requis de reproducteurs nécessaires varie à l'inverse du potentiel de reproduction, ceci dans les dix populations, entre 5 reproducteurs (AMM = 1,75 an) et 16 reproducteurs (AMM = 1,15 an) par ha.

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For fisheries management purposes, it is important to know the reproductive potential of Atlantic salmon (*Salmo salar*) in different rivers. Reproductive potential, defined as the average number of eggs per spawner, is a population trait which is used to determine the number of spawners needed to meet egg deposition requirements. Average eggs per spawner is difficult to estimate, however, because it is a function of several interrelated factors, including fish size, sea-age, fecundity, and sex ratio. Each of these reproductive traits varies among different stocks (Saunders 1981). Sea-age at maturity for example ranges from 1–3 yr, and varies both among populations from different geographic areas (Porter et al. 1986; Saunders 1986) and within populations from one year to the next (Ruggles and Turner 1973). Estimates of reproductive potential therefore requires extensive field sampling to determine all of the above reproductive traits for each individual stock of Atlantic salmon (see for example Marshall and Penney 1983; Randall 1984).

Because fecundity of Atlantic salmon, as with many species, increases with size of fish (Pope et al. 1961; Baum and Meister 1971; Thorpe et al. 1984), and because multi-sea-age salmon are predominantly females (Schaffer 1979), reproductive potential will generally increase with sea-age. Thus for any given egg deposition requirement, a population with an older mean sea-age will theoretically require fewer spawners per hectare than a population that matures at a younger age.

The objective of this paper is to define the relationship between sea-age and reproductive potential within and among populations of Atlantic salmon in eastern Canada. Interannual variability in reproductive traits is considered in two salmon populations in New Brunswick (Miramichi and Restigouche Rivers). Interpopulation variability is considered by comparing adult salmon from eight other populations with different maturation characteristics. A model is presented that can be used to estimate reproductive potential of salmon in rivers for which only the sea-age composition of spawners is known.

Methods

Terminology

Adult salmon that spawn for the first time after 1, 2, or 3 yr at sea are referred to as 1-sea-winter (1SW), 2 -sea-winter

(2SW), and 3-sea-winter (3SW) salmon. Iteroparous salmon are referred to as previous spawners (PS). Salmon that return after two or more years at sea, whether previous spawners or not, are collectively called multi-sea-winter (MSW) salmon.

Calculating Mean Sea-age and Reproductive Potential

Mean sea-age (MSA) of a spawning run of salmon was calculated as

$$(1)\quad \mathrm{MSA} = \mathrm{PSA1}\cdot(1\ \mathrm{yr}) + \mathrm{PSA2}\cdot(2\ \mathrm{yr}) + \mathrm{PSA3}\cdot(3\ \mathrm{yr}),$$

where PSA1 is the proportion of 1SW salmon in the spawning run, PSA2 is the proportion of 2SW salmon, and PSA3 is the proportion of 3SW salmon. Where possible, previous spawners were omitted from the estimate of mean sea-age. As will be seen later, these fish usually comprised less than 5% of the spawning run, and contributed less than 10% of the total egg deposition. For some populations however, previous spawners were included in the PSA2 or PSA3 terms (see later, Table 4).

Reproductive potential (RP) of a spawning run was calculated as

$$(2)\quad \mathrm{RP} = \sum_{i=1}^{4} [\mathrm{FF}_i\cdot\mathrm{PF}_i\cdot\mathrm{PSA}_i],$$

where FF_i is mean fecundity (number of eggs) of female salmon, at sea-age i, PF_i is proportion females to total fish at sea-age i, PSA_i is proportion of fish at sea-age i, to all fish.

So $\mathrm{PF}_i\cdot\mathrm{PSA}_i$ is the proportion of sea-age i females to all fish, and the sum of four terms is a weighted average of $\mathrm{FF}_i, \ldots \mathrm{FF}_4$ using those proportions as weights. Note that reproductive potential is an estimate of average eggs per fish in the spawning run, regardless of age or sex. Four age-groups were included in the equation, 1SW, 2SW, 3SW, and PS. Previous spawners were considered to be one age-group.

Data Sources

Intrapopulation Variation in Reproductive Potential

Variability in reproductive potential of adult spawners within populations among years was determined for the Miramichi River (1971 to 1983) and for the Restigouche River (1972 to 1980). Both rivers are located in northeast New Brunswick; a brief description of their salmon fisheries is given by Chadwick et al. (1985). For Miramichi River, salmon were sampled each year at a research trap located in the estuary at Millbank (trap description given by Ruggles and Turner 1973). Sampling included: one in five fish were live sampled for scales (for age determination), length (millimetres) and weight (0.1 kg); one in 10 fish was sacrificed for internal sexing. After early August each year, salmon were sexed on the basis of external characteristics. During the 13-yr period, 12 604 salmon were aged, 6523 salmon were sexed, and 2940 female salmon were measured for fork length.

In Restigouche River, a research trap was operated at Dalhousie, New Brunswick, from 1972 to 1980 (Peppar 1983). All salmon were measured (as for Miramichi samples) and sampled for scales. Sex ratios were determined by external sexing and from internal sexing of all salmon that became meshed in the trap. During the 8-yr period, 8672 salmon were aged, 2524 salmon were sexed, and 687 female salmon were measured for fork length.

For both the Miramichi and Restigouche Rivers, it was assumed that biological characteristics of trapped salmon were representative of the entire river.

Fecundity estimates of salmon were available for only 1 yr in Restigouche River and for 2 yr in Miramichi River. To determine the relationship between fecundity and female size, age and spawning history, 91 specimens were collected from Restigouche River in 1983 and 256 specimens were collected from Miramichi River, in 1982 and 1983. Specimens were collected from commercial salmon fishermen and anglers (both rivers) and from Millbank trap in the Miramichi. Fish were collected from June to September during their upstream migration. Samples represented all sea-ages: 1SW (n= 92); 2SW (n=195); 3SW (n=39) and PS (n=20). One specimen was of unknown sea-age.

Ovaries were placed in Gilson's fluid until ovarian tissue broke down, and were then transferred to 10% formalin to harden the eggs. Eggs in each sampled ovary were completely counted. Other information recorded for each fish included fork length (nearest centimetre), weight (nearest 0.1 kg), sampling location, date, gear, and age (from scales). Fecundity was recorded as total fecundity (total eggs per fish) and relative fecundity (eggs per kilogram). Length–fecundity relationships were calculated after both fork lengths and total fecundity were $\log_e$ transformed; the rationale for using this transformation is discussed by Pope et al. (1961) and Healey and Heard (1984).

Average eggs per female (FF in equation (2)) was calculated using mean fork lengths of female salmon (sea-age i) for each year and the length–fecundity relationship calculated for each river.

Interpopulation Variation in Reproductive Potential

In addition to the above information on the Miramichi and Restigouche Rivers, reproductive traits of salmon from eight other rivers were compared. These rivers included the Nepisiguit and Saint John in New Brunswick, the Margaree, St. Mary's and LaHave in Nova Scotia, and Western Arm, Middle and Conne in Newfoundland. Most of these rivers are assessed annually within the Anadromous and Catadromous Fishes Subcommittee of the Canadian Atlantic Fisheries Scientific Advisory Committee (CAFSAC), and the data were available in CAFSAC research documents (references given in Table 4).

Eggs per spawner for these populations were calculated using equation (2). Length–fecundity relationships were available for six of the eight stocks; for the remaining two (Margaree and Nepisiguit), an average relative fecundity of 1760 eggs$\cdot$kg^{-1} was used.

Although prepared for another reason, Porter et al. (1986) provided data that can be used to describe the theoretical relationship between the mean sea-age of spawners and reproductive potential of salmon in eastern Canada. Using data for seven hypothetical stocks ranging in mean sea-age from 1.0 to 3.0 yr, Porter et al. (1986) calculated the numbers of spawners required to produce 10 000 smolts in each of the stocks. Because the egg-to-smolt survival rate was assumed to be constant (0.02) among stocks, their results can easily be backcalculated to estimate eggs per spawner. Fecundity and size-at-age of spawners were kept constant among the seven stocks, and sex ratios were nearly constant, so that mean eggs per spawner was a direct function of sea-age. I compared this hypothetical relationship between eggs per spawner and sea-age to that calculated for the 10 populations for which actual reproductive traits were known.

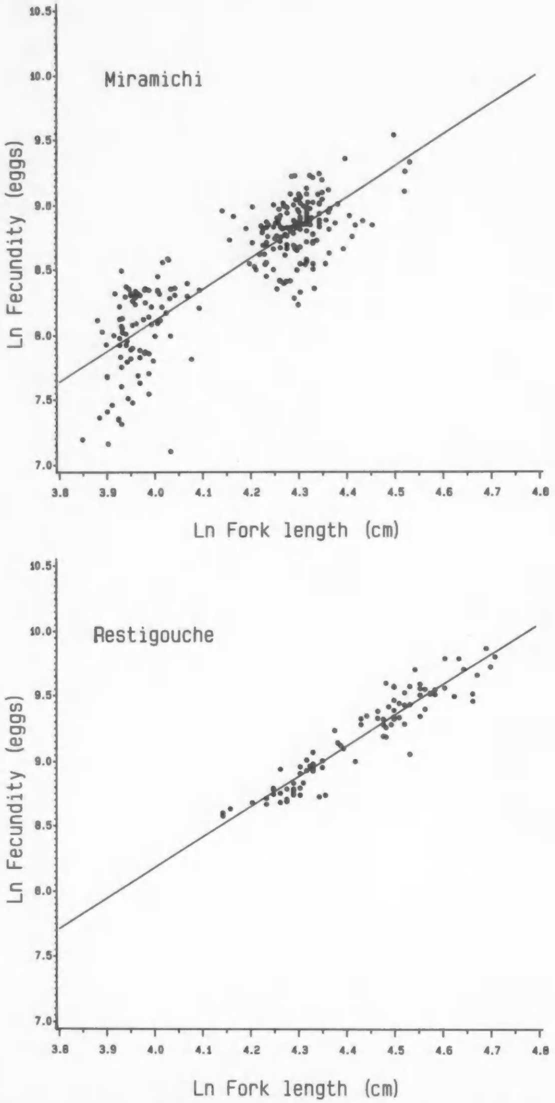

FIG. 1. Length–fecundity relationship for Atlantic salmon in the Miramichi (upper) and Restigouche Rivers (lower) New Brunswick. Regression equations are in Table 1.

Required Spawners

Numbers of salmon required for spawning (RS) for each of the 10 actual and seven hypothetical stocks were calculated as the total egg deposition requirements per hectare of rearing area divided by the average eggs per spawner (RP). Where estimates of RP were available for more than 1 yr (as for Miramichi and Restigouche Rivers), a mean RP for all years combined was used. For all rivers, a required egg deposition rate of 2.4×10^4 eggs per hectare was assumed. This deposition rate is tentatively accepted as an appropriate target for optimum salmon production in current CAFSAC assessments in eastern Canada. Note however that 2.4×10^4 eggs per hectare is a seeding rate which applies to fluvial rearing habitat only. For rivers in insular Newfoundland, where significant juvenile salmon production also occurs in lacustrine habitat (O'Connell 1986; O'Connell and Ash 1989), an appropriate target deposition rate has not yet been determined. The numbers of required spawners identified for the Newfoundland rivers in this paper applies only to fluvial habitat within these rivers, and are presented for comparative purposes only. They are not appropriate target egg depositions or required spawners for the entire watershed areas (i.e., fluvial plus lacustrine habitat) of these Newfoundland rivers.

Results

Fecundity of Salmon in the Miramichi and Restigouche Rivers

Fecundity of Atlantic salmon increased significantly with size in both the Miramichi and Restigouche Rivers (Fig. 1). For all sea-ages combined, length explained 72 and 89% of the changes in fecundity in the Miramichi and Restigouche samples, respectively (Table 1). Within sea-age groups, however, the correlation between length and fecundity was not as strong; length explained only from 8 to 70% of variability in fecundity of 1SW, 2SW, and 3SW salmon. For any given fork length, fecundity was highly variable, particularly for 1SW salmon in Miramichi River (Fig. 1).

For all sea-ages combined, regression coefficients were not significantly different between the Miramichi and Restigouche Rivers ($t = 0.06$, $P < 0.50$). However, elevations were significantly different ($t = -9.30$, $P < 0.01$), indicating Restigouche female salmon had significantly more eggs at a given fork length than Miramichi salmon.

Relative fecundities of salmon (eggs per kilogram) decreased with sea-age in both rivers (Table 1). Relative fecundities of 1SW and 2SW Miramichi salmon were not different (Duncan's multiple range test), but PS salmon had fewer eggs per kilogram than 1SW salmon. In the Restigouche sample, relative fecundity of 2SW salmon was significantly greater than 3SW and PS salmon, but there was no difference between the latter two groups.

Intrapopulation Variation in Mean Sea-age and Reproductive Potential

Miramichi river

From 1971 to 1983, MSA of salmon in the Miramichi River ranged between 1.06 (1981) and 1.54 (1977) yr. Age 1SW salmon were the most abundant, comprising 75% of the spawners, on average (Table 2). Age 2SW salmon comprised 21% of the spawning run on average, but the proportion varied considerably from one year to the next (Fig. 2). Age 3SW salmon and previous spawners were scarce, comprising < 1 and 3% of the population, respectively. The age composition of Miramichi salmon differed markedly among the 13 yr (Chi-square = 3793, $P < 0.01$).

In addition to age composition, reproductive potential is a function of female size at age and sex ratio (equation (2)), and each of these traits was variable among years. Age 1SW salmon were predominantly males (average 76%), while 2SW salmon were mostly females (89%), but sex ratios of both sea-ages varied significantly among years (rx2 Chi-square = 73.7 and 88.7 for 1SW and 2SW salmon, respectively, both significant at $P < 0.01$). Age 1SW salmon did not differ significantly in length among years, but 2SW salmon did (unbalanced ANOVA, $F = 9.78$, $P < 0.01$).

TABLE 1. Sea-age, mean fork length, mean fecundity (total eggs), and relative fecundity (eggs per kilogram) of Atlantic salmon in the Miramichi and Restigouche Rivers, New Brunswick. Regression coefficients for the regression of $\log_e$ fecundity on $\log_e$ length are also given. (SE of estimates given in parenthesis).

River	Sea-age (yr)	*n*	Mean fork length (cm)	Mean fecundity (No. eggs)	Relative fecundity (eggs·kg^{-1})	Regression statistics[a]			
						a	*b*	R^2	*F*
Miramichi	1SW	92	53.0 (0.3)	3230 (104)	2035 (62.0)	−4.5636 (2.6165)	3.1718 (0.6591)	0.20	23.15
	2SW	152	73.2 (0.3)	6939 (116)	1636 (26.0)	3.5030 (1.5045)	1.2395 (0.3505)	0.08	12.50
	PS	11	82.1 (2.6)	7676 (589)	1331 (76.8)	0.9212 (2.2052)	1.8164 (0.5008)	0.59	13.16
	All	256[b]	66.4 (0.7)	5665 (143)	1768 (30.2)	−1.3847 (0.3918)	2.3743 (0.0936)	0.72	643.29
Restigouche	2SW	43	74.2 (0.8)	7274 (205)	1600 (18.1)	−0.2049 (0.9240)	1.1101 (0.2147)	0.70	96.62
	3SW	38	92.4 (0.8)	12 606 (267)	1371 (17.1)	3.7813 (1.7700)	1.2491 (0.3911)	0.22	10.20
	PS	9	104.7 (2.0)	16 585 (716)	1306 (42.7)	—	—	—	NS
	All	91[c]	84.8 (1.3)	10 415 (379)	1475 (17.5)	−1.1862 (0.3872)	2.3423 (0.0874)	0.89	718.92

[a] Regression statistics, where a = intercept, b = slope, R^2 = coefficient of determination, and $F = F$ statistic for the correlation coefficient (all significant at $P<0.05$ or less).

[b] Includes 1 3SW salmon.

[c] Includes 1 salmon of unknown age.

TABLE 2. Reproductive traits of spawners in the Miramichi and Restigouche Rivers, New Brunswick. Data were collected from 1971 to 1983 for Miramichi salmon and from 1972 to 1980 for Restigouche salmon. FL = mean weighted fork length of female salmon (centimetres), PF = mean weighted proportion females and PSA is mean proportion at sea-age; *n* = sample size, and Range = the range of annual means (FL) or annual proportions (PF and PSA). Weighting was based on sample sizes from individual years.

	n	FL	Range	n	PF	Range	n	PSA	Range
Miramichi									
1SW	1000	52	51–53	4234	0.24	0.11–0.38	8481	0.75	0.44–0.92
2SW	1819	74	71–74	2058	0.89	0.70–0.95	3714	0.21	0.06–0.52
3SW	24	82	—	24	1.00	—	46	0.01	—
PS	97	81	—	207	0.46	—	363	0.03	—
MSW	1940	74	71–75	2289	0.85	0.69–0.91	4123	0.25	0.08–0.56
Restigouche									
1SW	40	52	—	1488	0.02	0.00–0.05	3150	0.39	0.22–0.56
2SW	380	76	73–77	699	0.54	0.28–0.75	3987	0.43	0.20–0.57
3SW	242	92	89–94	291	0.85	0.43–0.97	1264	0.14	0.08–0.31
PS	25	100	—	46	0.55	—	271	0.03	0.02–0.07
MSW	647	81	77–83	1036	0.63	0.49–0.70	5522	0.61	0.44–0.78

Despite significant annual variation in sex ratios and size of females, mean sea-age was the primary factor affecting the reproductive potential of salmon in the Miramichi River. Reproductive potential of the average salmon, including all age-groups and both male and female fish, ranged from 1123 (1981) and 3537 (1977) eggs per spawner, and it was directly correlated to the mean sea-age among years (Fig. 3; $R^2 = 0.88$, $n = 13$, $P < 0.01$). Age 1SW females produced an average of 2985 eggs (range 2877–3067), while the fecundity of 2SW females averaged 6694 eggs (6224–6955) (Table 3). On average, 2SW salmon contributed the most to annual egg deposition (64%), followed by 1SW salmon (29%), previous spawners (5%), and 3SW salmon (< 1%). Proportional contributions to total egg deposition from the different age-groups differed substantially among years (Table 3).

Restigouche river

Restigouche salmon were generally older and larger than Miramichi salmon (Fig. 2). From 1972 to 1980, mean sea-age ranged from 1.60 (1975) to 1.97 (1973) yr. Age 2SW salmon were usually the most abundant in the spawning run on average (43%), followed by 1SW salmon (39%), 3SW salmon (14%), and previous spawners (3%). Sea-age composition varied significantly from 1972 to 1980 ($r \times 4$ Chi-square = 1127, $P < 0.01$).

Age 1SW salmon were almost exclusively males (average 98%), 2SW salmon were 54% female, and 3SW salmon were predominantly females (85%). Sex ratios of both 2SW and 3SW salmon varied significantly among years ($r \times 2$ Chi-square = 67.4 and 28.9, respectively, both $P < 0.01$). Mean fork lengths of 2SW salmon ranged from 73.1 to 76.9 cm (Fig. 2), and between 89.4 and 93.5 cm for 3SW salmon (Table 2).

From 1972 to 1980, reproductive potential ranged between 2551 (1979) and 4936 (1973) eggs per spawner. During the 8-yr period, average eggs per spawner was correlated directly with mean sea-age, but the correlation was not as strong as in the Miramichi (Fig. 3; $R^2 = 0.61$, $n = 9$, $P < 0.02$). Age 2SW salmon contributed the majority of eggs (average 51%), 3SW salmon contributed 39%, previous spawners 8%, and 1SW salmon only 1%.

Interpopulation Variation in Biological Characteristics of Spawners

Four major differences were evident in the biological char-

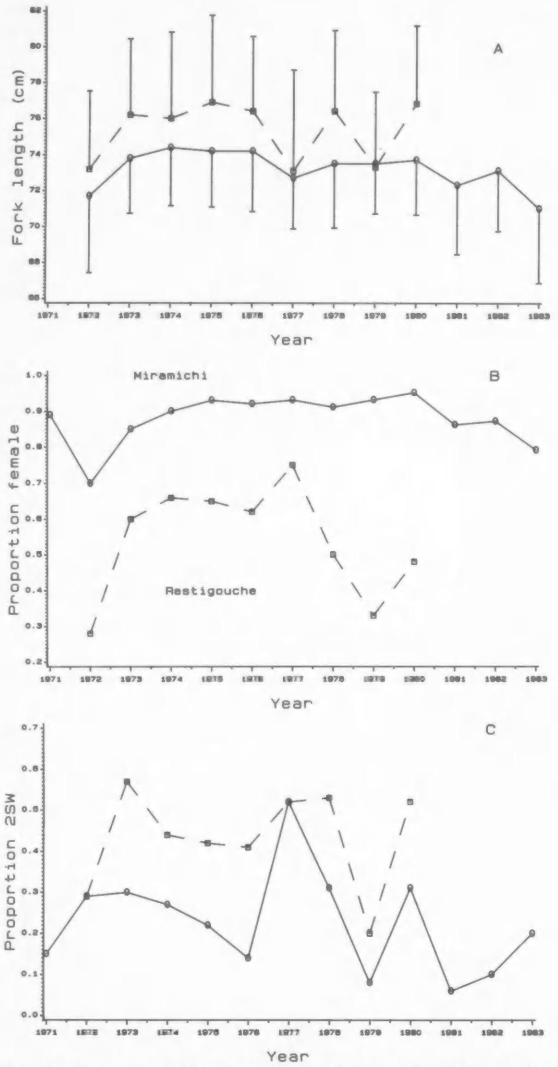

FIG. 2. Annual variability in (A) mean fork length, (B) proportion female, and (C) proportion of the total spawning run of 2SW salmon in the Miramichi (solid line) and Restigouche (dashed line) Rivers. Vertical lines in (a) indicate +1SD (Restigouche) and −1SD (Miramichi).

acteristics of spawners in the Miramichi and Restigouche Rivers (Tables 2 and 3): (1) age 1SW salmon were relatively less abundant, had a lower proportion of females, and contributed less to egg deposition in the Restigouche (1% of eggs) than in the Miramichi (29%); (2) correspondingly, age 3SW salmon were more numerous and contributed substantially more to egg deposition in the Restigouche (contributing 39 versus < 1% in the Miramichi); (3) mean sea-age at maturity was older for Restigouche salmon (1.76 yr) than for Miramichi salmon (1.24 yr); (4) reproductive potential was greater for the Restigouche (3635 eggs per spawner) than for the Miramichi (1982 eggs).

Differences in biological characteristics of spawners among populations of Atlantic salmon in eastern Canada were further emphasized when the eight other stocks were compared (Table 4). Mean sea-age in all populations ranged between 1.0 and 1.76, and mean reproductive potential ranged between 1482 and 4945 eggs per spawner.

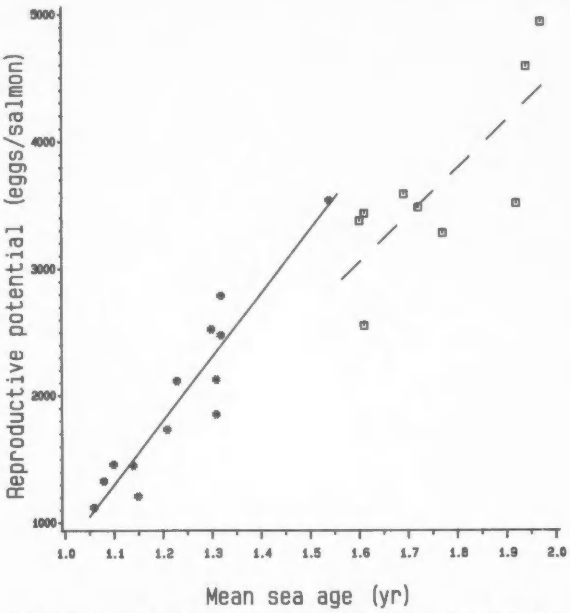

FIG. 3. Relationship between mean sea-age (years) and reproductive potential (eggs per spawner) in the Miramichi River (1971 to 1983, asterisks) and the Restigouche River (1972 to 1980, squares).

TABLE 3. Fecundity of Atlantic salmon of different sea-ages in the Miramichi and Restigouche Rivers, New Brunswick. Mean eggs per female (FF) was calculated using mean fork lengths of female salmon (sea age *i*) for each year (1971 to 1983 for Miramichi; 1972 to 1980 for Restigouche) and the length fecundity relationship calculated for each river (Table 1). Average proportional contribution to total egg deposition by each sea-age is also indicated.

	Mean eggs per female (FF)		Proportion contribution to total egg deposition	
	Overall mean (all years)	Range of means (among years)	Overall mean (all years)	Range (among years)
Miramichi				
1SW	2985	2877–3067	0.29	0.06–0.60
2SW	6694	6224–6955	0.64	0.30–0.90
3SW	8737	—	0.01	0.00–0.05
PS	8485	—	0.05	0.02–0.10
MSW	6822	6287–7112	0.71	0.37–0.94
Restigouche				
1SW	3193	—	0.01	0.00–0.02
2SW	7622	7091–7985	0.51	0.16–0.80
3SW	12 086	11 363–12 621	0.39	0.14–0.78
PS	14 634	—	0.08	0.03–0.17
MSW	8978	8009–9629	0.99	0.98–1.00

Data from Porter et al. (1986) indicated a direct and positive relationship between the mean sea-age (MSA) and reproductive potential (RP) in their seven hypothetical stocks (Fig. 4). The relationship was described by the power regression:

TABLE 4. Comparison of the biological characteristics and reproductive potential of Atlantic salmon in 10 rivers of eastern Canada. Required spawners per hectare are estimated assuming an egg deposition requirement of 2.4×10^4 eggs per hectare (see text).

River	Mean sea-age (yr)	Mean fork length (cm)	Proportion females	Eggs per spawner	Required spawners per hectare	Data sources
Restigouche	1.76	70	0.39	3635	6.6	This study
Margaree	1.75[a]		0.59	4945	4.9	Claytor and Leger (1986)
Saint John	1.57[a]		0.52	3781	6.4	Marshall and Penny (1983)
Nepisiguit	1.38[a]		0.37	2303	10.4	Lutzac (1984)
St. Mary's	1.30[b]	62	0.54	2365	10.2	Marshall (1986)
Miramichi	1.24	58	0.39	1982	12.1	This study
LaHave	1.15	57	0.39	1482	16.2	Cutting and Gray (1984)
Western Arm	1.00[c]	52	0.68	2304	10.4[d]	Chadwick et al. (1986)
Middle	1.00[c]	51	0.79	2204	11.0[d]	O'Connell (1986)
Conne	1.00[c]	53	0.76	2030	11.8[d]	Porter (1986) and Dempson et al. 1987

[a] Assumes all MSW salmon are 2SW and includes previous spawners.

[b] Includes previous spawners.

[c] Less than 5% of spawners are PS.

[d] For the three Newfoundland rivers, the numbers of required spawners (and the 2.4×10^4 eggs per hectare on which they are based) are applicable to fluvial habitat within these rivers only.

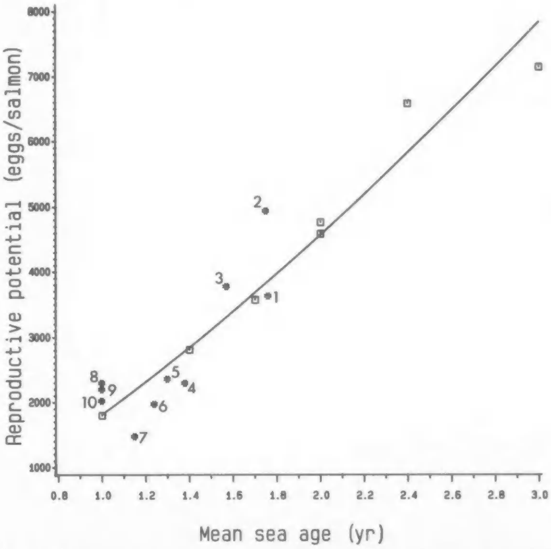

FIG. 4. Relationship between mean sea-age and reproductive potential in: (1) seven hypothetical salmon stocks ranging in MSA from 1.0 to 3.0 yr (calculated from data in Porter et al. 1986) (squares and regression line) and (2) for 10 salmon populations in eastern Canada (populations are identified in Table 4).

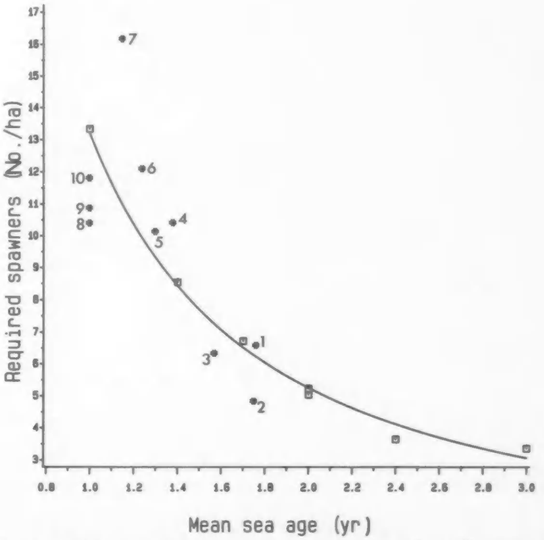

FIG. 5. Relationship between mean sea-age and numbers of spawners required to provide a deposition rate of 2.4×10^4 eggs per hectare. For further explanation, see caption for Fig. 4.

$$(3) \quad \text{RP (hypothetical)} = 1818.87\text{MSA}^{1.33} \; (R^2 = 0.98, F = 7.7, P < 0.05).$$

Mean sea-age and reproductive potential in the 10 actual populations (Table 4) were also significantly correlated (Fig. 4), but the coefficient of determination was lower:

$$(4) \quad \text{RP (empirical)} = 1831.26\text{MSA}^{1.30} \; (R^2 = 0.64, F = 7.7, P < 0.05).$$

Stocks that included significant numbers of MSW salmon (rivers 1–7, Table 4) had a high correlation between RP and MSA ($R^2 = 0.92$, $n = 7$, $P < 0.01$), while stocks that were predominantly 1SW fish (rivers 8–10) had more eggs per spawner than that predicted by Porter et al.'s data (Fig. 4). Analysis of covariance indicated that for all rivers combined, there was no significant difference between the regression coefficients or elevations of equations (3) and (4). This suggests that for salmon populations with mean sea-ages greater than 1.76 yr, reproductive potential may be extrapolated from equation (4); however, this should be verified with empirical data.

Required Spawners

Assuming a target egg deposition of 2.4×10^4 eggs, Porter et al.'s (1986) data indicated that from about three to 14 spawners are required per hectare, depending on the MSA of the spawning populations (Fig. 5). The relationship between required spawners (RS) and MSA was described by the equation

$$(5) \quad \text{RS (hypothetical)} = 13.21\text{MSA}^{-1.33} \; (R^2 = 0.98).$$

Note that RS is inversely proportional to RP (equation (3)). Required spawners from the 10 actual populations ranged between 4.9 and 16.2 fish per hectare (Fig. 5 and Table 4), as described by the equation

(6) $\mathrm{RS\ (empirical)} = 13.12\mathrm{MSA}^{-1.30}\ (R^2 = 0.64)$.

For populations of Atlantic salmon that have significant proportions of MSW salmon, most eggs come from these large spawners even if their relative abundance is low. The relationship between proportion of MSW salmon in the spawning run and proportion of eggs from MSW salmon (Fig. 6), indicates that if 40% of spawners are MSW, about 80% of eggs come from MSW salmon. If egg production from 1SW salmon are ignored in these populations, average eggs per spawner increases substantially, and required spawners are significantly less (compare Tables 4 and 5). For the seven MSW stocks in Table 5, required spawners ranged between only four and six fish per hectare, regardless of the variability of the other attributes of spawners.

Discussion

Reproductive potential (average eggs per spawner) of Atlantic salmon varied three-fold both within populations over time and among populations from different rivers in eastern Canada. All reproductive traits that determine eggs per spawner, however, were directly related to sea-age, including fecundity, length, and sex ratio. Therefore, reproductive potential and mean sea-age were significantly and positively correlated within and among populations. This correlation is potentially useful to assessment biologists: both reproductive potential and required spawners per hectare can be estimated for any Atlantic salmon population for which the sea-age composition is known (Figs. 4 and 5).

Fecundity estimates for Miramichi and Restigouche salmon in this study indicate potential fecundity only, and they are probably overestimates. Ovaries were collected during the spawning run from June to September; because all salmon spawn in late October–early November in these rivers, egg maturation would continue for another 1–4 mo after samples were collected. Atretic oocytes are known to occur in salmonids (Bagenal 1978), and my egg counts would include atretic oocytes that would subsequently be resorbed. Prouzet et al. (1984) provided evidence that about 30% of oocytes in a population of salmon from France did not mature. Thus, the fecundity estimates for Miramichi and Restigouche salmon are approximations only.

Female salmon from Restigouche River had significantly more eggs at a given length than Miramichi salmon. Stock-specific fecundities have been found in other studies of Atlantic salmon (Pope et al. 1961; Glebe et al. 1979) and fecundity is considered to be a genetic trait unique for each stock (Saunders 1981). Total fecundities of Miramichi and Restigouche salmon were however within the range of fecundities reported from other rivers in eastern Canada and elsewhere (Table 6).

Although many studies have been done on fecundity of Atlantic salmon from different areas throughout their geographic range (Table 6), there have been no studies on interannual variation within individual populations. If temporal variation is significant, as it is in other salmonids such as chinook salmon (Healey and Heard 1984), it would contribute to variability in the sea-age/reproductive potential relationship as well.

In spawning populations that mature at different sea-ages, large MSW salmon contribute proportionally more to total egg deposition than 1SW salmon (Fig. 6). The difference in reproductive potential between sea-ages can be substantial: in the Miramichi and Restigouche Rivers, for example, MSW salmon produce two to three times more eggs per fish than 1SW salmon (Table 3). This emphasizes the importance of MSW salmon in spawning populations with multiple sea-ages at maturity. Because of the high reproductive potential of these fish, it requires considerably less spawning biomass and numbers to meet spawning requirements if it is assumed that all eggs come from MSW salmon (Randall 1985). In the Miramichi River, for instance, which has a total rearing area of about 5500 ha, about 66 550 spawners are required if all sea-ages are included (5500 × 12.1; Table 4), but only 23 100 spawners are required if MSW salmon only are utilized (5500 × 4.2; Table 5). (Note, however, that because MSW salmon are predominantly female, additional spawners would be required if a 1:1 sex ratio at spawning were desired (Randall 1985)). A management strategy designed to maximize both spawning levels and fishing yield would favour harvest of 1SW salmon and protection of MSW salmon.

Precise estimates of reproductive potential require annual sampling to determine the biological characteristics of adult spawners for each specific stock. Extensive field sampling is costly, however, and it is often not feasable. The relationship between sea-age and reproductive potential defined previously (Tables 4 and 5) provides a method for estimating eggs per spawner with a minimum field effort (random sampling for age composition). In estimating the potential egg deposition of a sockeye salmon (*Oncorhynchus nerka*) population over several years, Mathisen and Gunnerod (1969) found that the greatest variance component was in estimating population size, while variance in reproductive potential (proportion females and fecundity) was relatively less. For Atlantic salmon stocks in eastern Canada, where annual assessments are required but

TABLE 5. Biological characteristics and reproductive potential of MSW salmon in seven river populations.

River	Proportion MSW	Mean fork length (cm)	Proportion females	Proportion eggs from MSW	Eggs per MSW spawner	Required spawners per hectare
Restigouche	0.61	81	0.63	0.99	5578	4.3
Margaree	0.75		0.75	0.98	6482	3.7
Saint John	0.57		0.87	0.98	6592	3.6
Nepisiguit	0.38		0.71	0.89	5373	4.5
St. Mary's	0.22	78	0.63	0.46	4655	5.2
Miramichi	0.25	74	0.85	0.71	5703	4.2
LaHave	0.15	73	0.73	0.43	4247	5.7

TABLE 6. Comparative fecundity of Atlantic salmon from different rivers in eastern Canada, Maine (United States), and the United Kingdom.

Location	*n*	Fork length (range in cm)	Total fecundity 50	70	90	Authority
Canada						
Restigouche	91	63–111		6406	11 541	This study
Miramichi	256	47–93	2707	6018	10 929	This study
Big Salmon	28	50–88	2473	6722		Glebe et al. 1979
Saint John	121	51–102	2609	5365	11 034	Marshall and Penney 1983
St. Mary's	53	48–98	2387	5199	11 324	Marshall 1986
LaHave	53	52–95		5539	11 222	Cutting and Jefferson 1986
Middle	102	38–62	2716			O'Connell 1986
Conne	30	46–56	2323			Dempson et al. 1987
United States						
Maine[a]	133	66–100		6593	12 775	Baum and Meister 1971
United Kingdom						
Almond	104	55–91		6242	10 740	Thorpe et al. 1984
Several[b]	402	48–95	2422	5314	9554	Pope et al. 1961

[a] Includes two rivers.
[b] Includes six rivers.

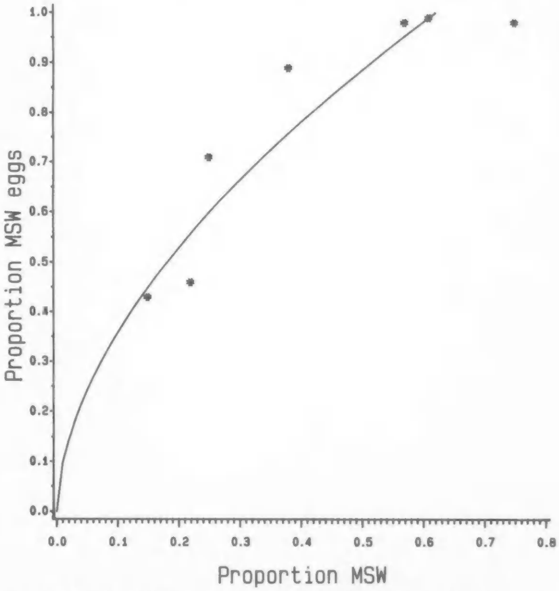

FIG. 6. Relationship between proportion MSW salmon in the spawning run and proportion of total egg deposition from MSW salmon for seven salmon populations in eastern Canada. The relationship is described by the power regression: proportion MSW eggs = 1.31 (proportion of MSW salmon)$^{0.56}$ ($R^2 = 0.86$). Data are in Table 5.

resources for field sampling are limited, it may be desirable to expend more field effort to estimate population size than to determine reproductive traits of spawners per se. Information on reproductive traits of populations with mean sea-ages > 1.76 yr is needed, however, to determine if the above relationship between sea-age and reproductive potential can be extrapolated to these populations.

Acknowledgements

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References

BAGENAL, T. B. 1978. Aspects of fish fecundity, p. 75–101. *In* S. D. Gerking [ed.] Ecology of freshwater fish production. Blackwell Scientific Publications, London, U.K.

BAUM, E. T., AND A. L. MEISTER. 1971. Fecundity of Atlantic salmon (*Salmo salar*) from two Maine rivers, J. Fish. Res. Board Can. 28: 764–767.

CHADWICK, E. M. P., D. R. ALEXANDER, R. W. GRAY, T. G. LUTZAC, J. L. PEPPAR, AND R. G. RANDALL. 1985. 1983 research on anadromous fishes, Gulf Region. Can. Tech. Rep. Fish. Aquat. Sci. No. 1420: xi + 69 p.

CHADWICK, E. M. P., R. G. RANDALL, AND C. LÉGER. 1986. Ovarian development of Atlantic salmon (*Salmo salar*) smolts and age at first maturity, p. 15–23. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

CLAYTOR, R. R., AND C. LÉGER. 1986. Assessment of Atlantic salmon, *Salmo salar*, in the Margaree River, Nova Scotia, 1986. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 86/93.

CUTTING, R. E., AND R. W. GRAY. 1984. Assessment of the status of Atlantic salmon stocks of the LaHave River, Nova Scotia. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 84/40.

CUTTING, R. E., AND E. M. JEFFERSON. 1986. Status of the Atlantic salmon of the LaHave River, Nova Scotia, in 1985 and forecast of returns in 1986. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 86/17.

DEMPSON, J. B., T. R. PORTER, AND G. FUREY. 1987. Assessment of the Atlantic salmon population of Conne River, Newfoundland, 1987. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 87/104.

GLEBE, B. D., T. D. APPY, AND R. L. SAUNDERS. 1979. Variation in Atlantic salmon reproduction traits and their implications to breeding programs. International Council for the Exploration of the Sea C. M. 1979/M:23.

HEALEY, M. C., AND W. R. HEARD. 1984. Inter- and intra-population variation in the fecundity of chinook salmon (*Oncorhynchus tshawytscha*) and its relevance to life history theory. Can. J. Fish. Aquat. Sci. 41: 476–483.

LUTZAC, T. G. 1984. Assessment of the Nepisiguit River salmon stock in 1983. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 84/2.

MARSHALL, T. L. 1986. Estimated spawning requirements and indices of stock status of Atlantic salmon in the St. Mary's River, Nova Scotia. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 86/22.

Marshall, T. L., and G. H. Penney. 1983. Spawning and river escapement requirements for Atlantic salmon of the Saint John River, New Brunswick. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 83/66.

Mathisen, O. A., and T. Gunnerod. 1969. Variance components in the estimation of potential egg deposition of sockeye salmon escapements. J. Fish. Res. Board Can. 26: 655–670.

O'Connell, M. F. 1986. Atlantic salmon (*Salmo salar* L.) egg deposition potential and annual smolt production for three river systems characterized by lacustrine habitat in insular Newfoundland. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 86/85.

O'Connell, M. F., and E. G. M. Ash. 1989. Atlantic salmon (*Salmo salar*) smolt production in a Newfoundland river system characterized by a lacustrine habitat. Int. Revue. Ges. Hydrobiol. 74: 73–82.

Peppar, J. L. 1983. Adult Atlantic salmon (*Salmo salar*) investigations, Restigouche River systems, New Brunswick, 1972–80. Can. Man. Rep. Fish. Aquat. Sci. No. 1695: viii + 33 p.

Pope, J. A., D. H. Mills, and W. M. Shearer. 1961. The fecundity of Atlantic salmon (*Salmo salar* Linn.). Freshwater and Salmon Fisheries Research Rep. 26. Department of Agriculture and Fisheries for Scotland, Edinburgh.

Porter, T. R. 1986. Assessment of the Atlantic salmon stock of Conne River, Newfoundland. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 86/6.

Porter, T. R., M. C. Healey, and M. F. O'Connell. 1986. Implications of varying the sea-age at maturity of Atlantic salmon (*Salmo salar*) on yield to the fisheries, p. 110–117. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

Prouzet, P., P. Y. LeBail, and M. Heydorff. 1984. Sex ratio and potential fecundity of Atlantic salmon (*Salmo salar* L.) caught by anglers on the Elorn River (Northern Brittany, France) during 1979 and 1980. Fish. Manage. 15: 123–130.

Randall, R. G. 1984. Number of salmon required for spawning in the Restigouche River, N.B. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 84/16.

1985. Spawning potential and spawning requirements of Atlantic salmon in the Miramichi River, New Brunswick. Canadian Atlantic Fisheries Scientific Advisory Committee Research Document 85/68.

Ruggles, C. P., and G. E. Turner. 1973. Recent changes in stock composition of Atlantic salmon (*Salmo salar*) in the Miramichi River, New Brunswick. J. Fish. Res. Board Can. 30: 779–786.

Saunders, R. L. 1981. Atlantic salmon (*Salmo salar*) stocks and management implications in the Canadian Atlantic provinces and New England, USA. Can. J. Fish. Aquat. Sci. 38: 1612–1625.

1986. The scientific and management implications of age and size at sexual maturity in Atlantic salmon (*Salmo salar*), p. 3–6. *In* D. J. Meerburg [ed.] Salmonid age at maturity. Can. Spec. Publ. Fish. Aquat. Sci. 89.

Schaffer, W. M. 1979. The theory of life-history evolution and its application to Atlantic salmon. Symp. Zool. Soc. London 44: 307–326.

Thorpe, J. E., M. S. Miles, and D. S. Keay. 1984. Developmental rate, fecundity and egg size in Atlantic salmon, *Salmo salar* L. Aquaculture 43: 289–305.

A New Cetacean from the Late Eocene La Meseta Formation, Seymour Island, Antarctic Peninsula

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The new genus and species ***Llanocetus denticrenatus*** is described based on a cranial endocast and portion of a dentary fragment bearing two teeth from the Eocene La Meseta Formation, Seymour Island, Antarctic Penninsula. ***Llanocetus*** new genus is designated the type genus for the new family **Llanocetidae**. The Llanocetidae is placed within the new infraorder **Crenaticeti**, co-ordinate with the new Mysticete infraorder **Chaeomysticeti.** *Llanocetus denticrenatus* new species shows relationship with both Archaeoceti and Mysticeti. Revision of archaeocete and mysticete classification includes proposal of the new taxa **Kekenodontinae**, **Mammalodontidae**, and the new ranks **Protocetoidea**, **Remingtonocetoidea**, **Basilosauroidea**, and **Eschrichtioidea.** The endocast shows evidence of intracranial retial hypertrophy, taken as evidence of enhanced diving abilities. The large deeply notched teeth are widely spaced on the lower jaw. I hypothesize that filter feeding in whales arose first in species in which teeth with deep notches, forming widey spaced tubercles, comprised part of a straining apparatus analogous to the serrated teeth of the phocid seal *Lobodon carcinophagus.*

Un nouveau genre et une nouvelle espèce, ***Llanocetus denticrenatus***, sont décrits à partir d'un moulage crânien interne et d'un fragment de mandibule portant deux dents qui provient de la formation La Meseta de l'Éocène de l'île Seymour, dans la péninsule arctique. Le nouveau genre ***Llanocetus*** est désigné le genre type de la nouvelle famille des **Llanocetidae**, elle-même placée dans le nouveau infraordre **Crenaticeti**, un coordonné du nouveau infraordre **Chaeomysticeti** du sous-ordre Mysticeti. *Llanocetus denticrenatus* est apparenté aux sous-ordres Archaeoceti et Mysticeti. Un examen de la classification de ces deux sous-ordres a généré la proposition des nouveaux taxons **Kekenodontinae** et **Mammalodontidae** ainsi que des nouveaux rangs **Protocetoidea**, **Remingtonocetoidea**, **Basilosauroidea** et **Eschrichtioidea.** Le moulage interne montre des signes d'hypertrophie réticulaire intracrânienne considérée comme un indice de meilleures capacités de plongée. Les dents, grosses et fortement crénelées, sont très espacées sur la mandibule inférieure. L'auteur émet l'hypothèse que l'alimentation par filtration est tout d'abord apparue chez les espèces de baleines dont les dents présentaient de profondes crénelures, formant des tubercules très espacés, et faisaient partie d'un appareil de filtrage analogue aux dents crénelées du phocidé *Lobodon carcinophagus.*

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Whales were known to the earliest explorers and natural historians, so the mammalian order Cetacea was comparatively well-characterized in the mid 18th century. By the early 19th century, fossils representing many different kinds of whales had been discovered and the broad nature of cetacean diversity recognized. By the mid to late 19th century, the Archaeoceti, Odontoceti, and Mysticeti were named and ranked as suborders, and a debate began which continues to this day. The question was whether these clades were closely related, or whether the Mysticeti and possibly even the Odontoceti were independently evolved from a non-archaeocete, terrestrial mammalian ancestor. The fossil record was and remains insufficient to resolve the issue, since there is a lack of fossils representing different species of graded morphological stages from superposed rocks of the appropriate age to show transitions between archaeocetes and the two suborders of living whales. The debate of necessity had to rely upon hard anatomy characters in comparisons with and to the archaeocetes, and upon hard and soft anatomy characters in comparisons between living species.

Weber (1904, 1928) framed a well reasoned argument for a monophyletic origin of odontocetes and mysticetes from archaeocetes. Others (summarized in Kellogg 1936; Barnes and Mitchell 1978) have argued that Cetacea are polyphyletic. The most recent authoritative arguments that the archaeocetes are not ancestors to living whales (Kleinenberg 1958, 1959; Yablokov 1964, 1965; Agarkov and Khomenko 1984) have been countered with persuasive arguments that the observed differences are just as adaptive as the similarities and that monophyly is the simplest explanation of the known facts (Zhemkova 1965, 1971; Van Valen 1968; Mchedlidze 1977; Barnes and Mitchell 1978; McLeod et al. 1989). Barnes and Mitchell (1978) evaluated fossil evidence and concluded that Dorudontinae may contain suitable morphological ancestors for both the Odontoceti and the Mysticeti. Cytogenetic evidence (Arnason 1974a,b) also has been used in support of monophyly, but such use has been critized (Haiduk et al. 1981; Arnason 1982).

The purpose of this paper is to describe fragmentary but important fossil remains of a new genus and species of whale that represents a morphological stage between Archaeoceti and Mysticeti. The new species is from Late Eocene rocks. Thus, it is of the appropriate age to be contemporaneous with but near the end of the dorudontine archaeocete radiation, and it antedates the time of occurrence of the earliest known mysticetes in Oligocene rocks. It is neither an archaeocete nor a mysticete whale according to the long-standing, simple and useful definitions of those taxa, so a new family and infraorder within mysticeti is erected to accommodate the new species.

Materials and Methods

Materials examined include relevant specimens of early Tertiary and other cetaceans in the following institutions (abbreviations in parentheses): British Museum (Natural History), London (BM(NH)); Geological Museum, Cairo (CGM); Geology Department, Melbourne University, Melbourne (MUDG); Natural History Museum of Los Angeles County, Los Angeles (LACM); Museum of Comparative Zoology, Harvard University, Cambridge (MCZ); Museum of Palaentology, University of California, Berkeley (UCMP); National Museum of Canada, Ottawa (NMC); United States National Museum of Natural History, Smithsonian Institution, Washington (USNM); National Museum of New Zealand, Wellington (NMNZ); National Museum of Victoria, Melbourne (NMV); Otago Museum, Dunedin (OM); Peabody Museum of Natural History, Yale University, New Haven (YPM); University of Canterbury, Christchurch; and Museum of Natural History, University of Oregon (UOMNH).

Holotypes or other hypodigm material of the following taxa were examined:

Aetiocetus cotylalveus Emlong 1966, UOMNH 26351; *Chonecetus sookensis* Russell 1968, NMC 12095, *Dorudon intermedius* (Dart) 1923, BM(NH) M-10173; *Dorudon*(?) *sensitivus* (Dart) 1923, BM(NH) M-12123; *Kekenodon onamata* Hector 1881, NMNZ Ma.306; *Mammalodon colliveri* Pritchard 1939, MUGD 1874, NMV P-17535; *Mauicetus parki* Benham 1937, OU 11573; *Mauicetus waitakiensis* Marples 1956, OM C.62.2; *Mauicetus brevicollis* Marples 1958, OM C.62.3 (=G.48.8); *Pappocetus lugardi* Andrews 1920, BM(NH) M-11414; and *Prozeuglodon isis* (Andrews) 1904, CGM 10208, CGM 9319. Also, referred specimens, casts, or other representations of most other relevant early Tertiary archaeocete and mysticete taxa were examined. Specimens of all living mysticete species were examined. In addition, a cross-section preparation 77-cm anterior of the condyle and thus anterior to the coronoid process of the 122-cm long right mandible was taken from a specimen of *Balaenoptera acutorostrata* (EDM specimen D62-72, 563 cm long female, taken 18 VI 1972, Dildo whaling station, Newfoundland). This was used to compare with the cross-sectional anatomy of the fossil fragment. Points of dentary structure and relationships were checked with a dissection of the head of a 295-cm long male fetus of *Balaenoptera physalus* (no. WF-2) from a 1925-cm long female landed 5 VIII 1972, Williamsport whaling station, Newfoundland (EDM specimen W40-72). Photographs (Fig. 1) were taken of the fossil specimens coated with ammonium chloride.

Systematics

Class Mammalia Linneaus 1758

Order Cetacea Brisson 1762 (= Cete Linnaeus 1758)

Infraorder **Crenaticeti**, new infraorder

Diagnosis:

Same as that of the genus and family until other genera and families are described.

Included families:

Llanocetidae, until others are referred or described.

The etymology refers to the notched structure (L. *crenatus*, notched, toothed) of the tooth crowns in the type species of the genus *Llanocetus*, and cetacean (*L. cetus*, whale).

Family **Llanocetidae**, new family

Type genus:

The type genus of the family Llanocetidae is *Llanocetus* new genus.

Diagnosis:

That of the genus until other genera are described.

Included genera:

Llanocetus new genus.

Genus ***Llanocetus***, new genus

Etymology:

Llano is a patronym in honor of Dr. George A. Llano, formerly of the National Science Foundation's Office of Polar Programs in Washington, D.C. who facilitated whale studies on the r/v *Hero* and who helped make these fossils available to me for study; combined with cetus (L., whale).

Type species:

Llanocetus denticrenatus new species

Diagnosis:

A medium sized cetacean with a skull approximately the size of the living *Balaenoptera acutorostrata* or of the larger archaeocetes, with functional teeth in both jaws in adult stage, teeth widely spaced along jaw, posterior end of tooth row apparently external of dorsal midline of dentary, bones roofing the braincase apparently telescoped with occipital shield sloping forward from vertical at an angle of approximately 50° and extending dorsoanteriorly over the cranium. Mandibular ramus large, massive, and deep, with small mandibular canal located near ventrointernal margin of ramus in cross-section, pronounced dorsal margin projected as a crest internal to the posterior end of the tooth row, body of mandibular ramus without highly excavated mandibular canal and with cortex comprised of pachyostotic bone with relatively small medulla of cancellous, not spongy, bone. Dentary with multicusped or denticulated functional teeth having nonconical crowns, large posterior cheekteeth double rooted with pronounced cusps, roots widely separated, greatest diameter proximally near crown, accessory cusps on crown shaped as nearly round (in cross-section) tubercles with small carina anteriorly and posteriorly, broadly open V-shaped cleft or notch between tubercles approximating in size the adjacent tubercle (if reversed), tubercles splayed anteroposteriorly around entire half circumference of crown, crown reduced, narrower than roots, with much reduced cingula. Endocast with olfactory peduncle, ethmoturbinal bones apparently present, inferred retial tissue massed anteriorly and dorsal to cerebrum not posteriorly and dorsal to cerebellum, 'Masses annexes' of Gervais large and shaped like those in living Mysticeti, thus cranial hiatus large. Endocast shows skull had broad foramen magnum, condyles apparently directed posterodorsally, basicranium broad laterally and short anteroposteriorly; bony remnant of interdigitations dorsoanteriorly indicate skull telescoped, cranial cavity high and wide but anteroposteriorly short.

Llanocetus denticrenatus, new species

Holotype:

Portion of right mandibular ramus, with two posterior cheekteeth in situ. The tubercles on the anterior edge of the anterior tooth are broken off. Specimen USNM 183022 (Figs. 1–3) was originally in a block of dark gray, fine-grained sandstone matrix before preparation. A fragment of maxillary bone bearing a fragmentary cheektooth is preserved with fragments of the crown of the opposing posterior lower cheektooth in situ in matrix. An isolated fragmentary cheektooth has only part of the crown with one denticle preserved. Some bone fragments with clean breaks but no fits were also collected, but are not readily determinable.

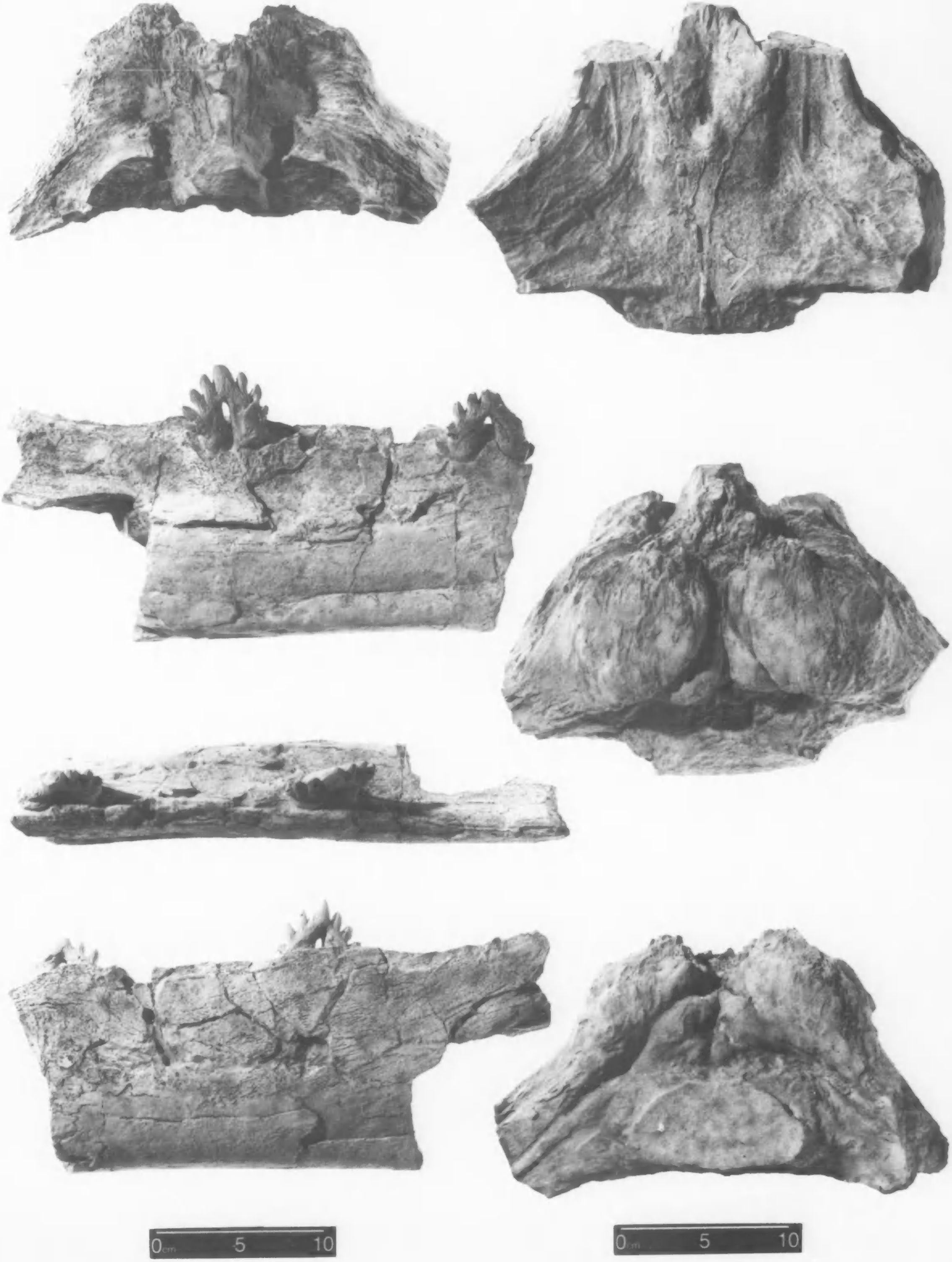
Referred material:

Complete natural matrix cranial endocast of dark gray fine-grain sandstone, with small remnants of bone adhering in crevices and concavities. Specimen USNM 183022 (Figs. 1,4), very probably from same individual as mandibular ramus.

Type locality:

In Unit III of the La Meseta Formation, associated with penguin fossils (Zinsmeister and Camacho 1982), northwestern Seymour Island, northern Antarctic Peninsula, approximately 64°14.85′S latitude, 56°37.42′W longitude (cf. Geo-

FIG. 1. *Llanocetus denticrenatus* new genus and species, holotype and referred specimen, USNM 183022. Clockwise from upper left: anterior, ventral, dorsal, and posterior views of natural cranial endocast; internal, dorsal, and external views of portion of right dentary.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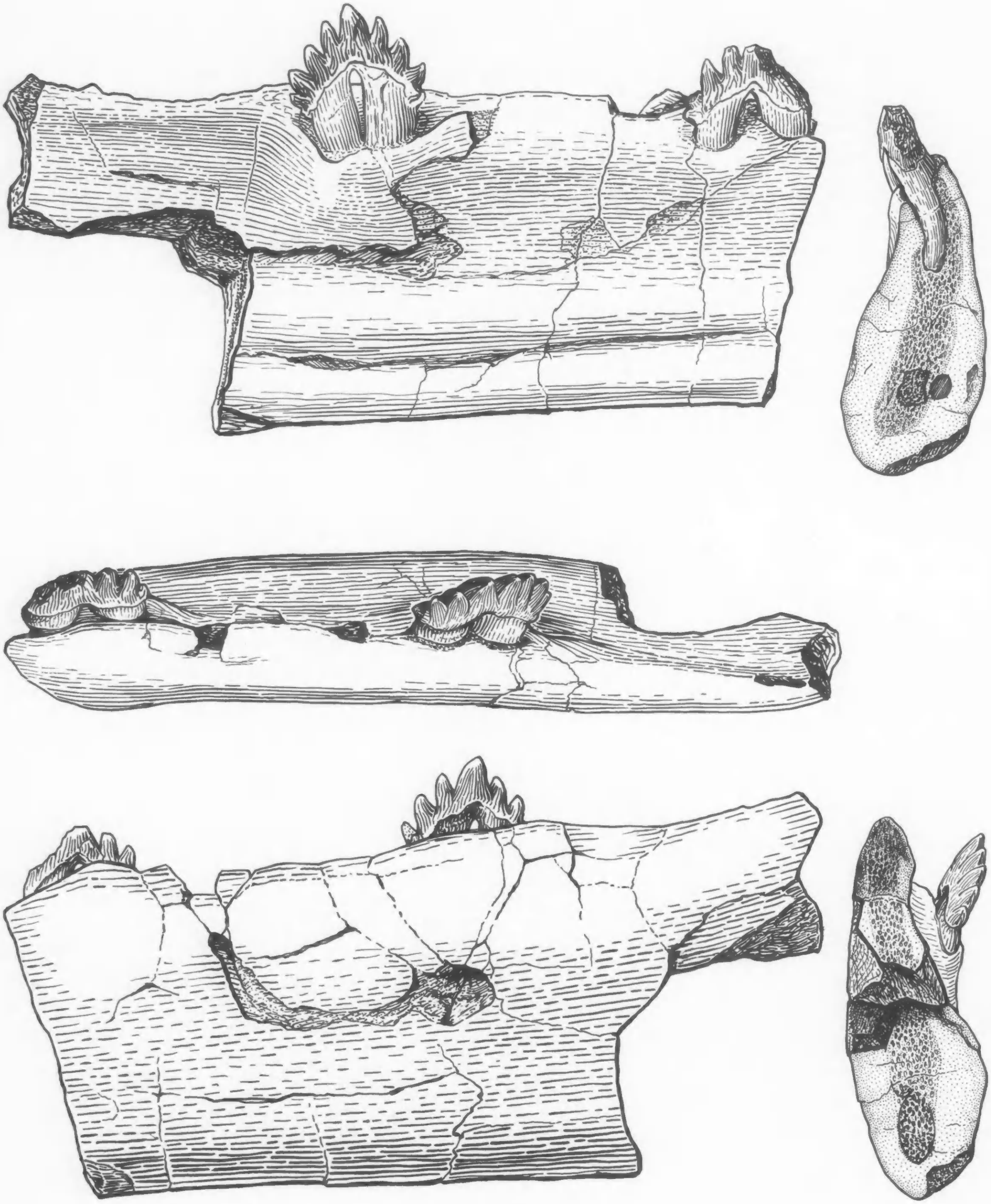

FIG. 2. *Llanocetus denticrenatus* new genus and species, portion of right dentary of holotype, USNM 183022. Clockwise from upper left: external, anterior, posterior, internal, and dorsal views. Specimen as preserved is 32.3 cm long.

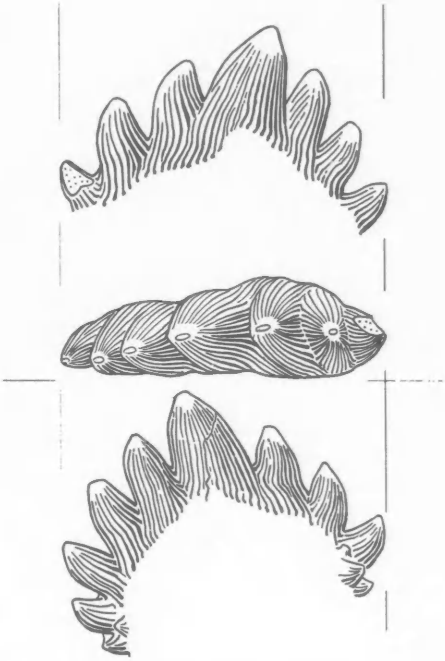

FIG. 3. *Llanocetus denticrenatus* new genus and species, enamel crown of posterior right cheektooth, USNM 183022. Top to bottom: medial, dorsal, and lateral views, approximately natural size.

logic Map of Seymour Island, folding map in pocket of Feldman and Woodburne, 1988). "The homogeneous medium to fine-grained, moderately to well-sorted sand points to shallow-marine or strandline conditions; these beds contain vertebrate remains" (Elliot and Trautman 1982). These authors also conclude that the La Meseta Formation sedimentary units were deposited in deltaic environments, with the lithofacies of Unit III representing possible lagoonal deposits protected from wave action by sand and gravel bars.

Collector:

Geologists from Ohio State University and Northern Illinois University were invited by the Instituto Antarctico Argentino to take part in their 1974–75 field work on Seymour Island. "A small amount of fossil penguin, shark, and whale bone material was collected… . All material collected occurred as float, and no attempt was made to quarry or to bring back large bulk samples. A small whale skeleton (*Zeuglodon*)(?) was found, however; fragments of the lower jaw containing teeth should yield valuable information concerning the whale's early evolution" (Elliot et al. 1975). It is my understanding that the endocast occurred as float but came from the same "small whale skeleton."

Age:

Woodburne and Zinsmeister (1984) reviewed data from a variety of dinoflagellate, mollusk, penguin, shark, and whale fossils indicating a Late Eocene age of the La Meseta Formation, with the upper part perhaps being as young as Early Oligocene. The La Meseta Formation is now regarded as Late Eocene in age, according to intepretations of the invertebrate faunas, with the lower portion as old as Middle Eocene according to interpretation of dinoflagellate cysts (Elliot 1988, and references therein cited).

Diagnosis:

That of the genus until other species are described.

Description of holotype — Portion of right dentary:

The piece of right mandibular ramus (USNM 183022) as preserved is 32.3 cm in length along the dorsal margin, and 22.0 cm along the ventral margin of the ramus. The size, degree of ossification, and surface texture of the bone indicate that it is from an adult individual. Its identification as right is based upon curvature, with the internal surface less strongly convex than the external surface. The mandibular canal was probably situated within the ventrointernal, cross-sectional quadrant of the ramus. (Alternatively, if the species were like an archaeocete, the posterior teeth would lie internal to the ascending crest leading posteriorly to the coronoid process. If this were the case, the cross-sectional shape and longitudinal curvature would not be appropriate. The following description assumes it is the right dentary.) Two nearly intact double-rooted teeth are located in situ, 15.2 cm apart on centers (diastema 10.7 cm), external or labial to the sharp dorsal margin of the mandible. The longitudinal space between the roots of the two teeth is 10.6 cm, less than the 11.3 cm from the root of the rear tooth to the posterior end of the ramus as preserved. Thus these may be the two most posterior teeth in the tooth row. The ramus is massive.

In cross-section the ramus is in the form of an irregular ovoid, widest in the lower half, with a convex lingual side and a sinuous (convexoconcave) labial side. At the anterior break it is 5.1 cm wide and 11.5 cm deep; at the posterior (ventral) break, 5.6 and 13.5 cm, respectively. I could not certainly locate the mandibular canal in this preparation; its tentative location is shown in Fig. 2. The anterior cross-section shows a medulla of densely spongy bone and a cortex of compact bone. The center of the ramus is nearly filled with cancellous bone with thick trabeculae. The compact bone comprising the cortex of the ramus appears to be thickest and pachyostotic on the sides and bottom. The dorsal margin of the ramus projects into a longitudinal, sharp-edged crest. It is not pachyostotic. The posterior cross-section shows the compact cortex of lamellar, pachyostotic bone to be up to 1.5 cm thick on the lingual side and 2.0 cm thick on the labial side, and 1.3 cm thick on the ventral margin. The cancellous medulla is only 20 mm across at the level where the ramus is widest at 56.8 mm.

The internal or lingual surface of the ramus is dorsoventrally convex and longitudinally concave. The surface is broken and pitted, but the main features are readily ascertained. The slight longitudinal curvature is reflected in an arc of 3.5 mm for a chord 23.0 cm long on the medial surface. The ventral half of this surface is slightly ornamented with relatively large rugae and pits of very small (ca. 0.3 mm) relief. The dorsal half of the medial surface is more heavily ornamented with longitudinally oriented rugae (6–7 per cm measured normal to their axes) and small pits. The ventral margin is slightly concave, arched 2.4 mm over a chord distance of 21.1 cm.

The external or labial surface is concave dorsally and strongly convex ventrally. The surface is somewhat distorted by longitudinal cracks and pits. The ornamentation appears to be similar to that of the lingual surface, as far as can be ascertained. There are broad, shallow, concave excavations on the dorsoexternal surface anterior and posterior to the alveolus for the posterior tooth. The tooth thus sits in an alveolus on a ledge, off to the external side from the uninterrupted line of the dorsal margin of the ramus. The anterior tooth is likewise off to the external side of this margin but the alveolar margins do not project out so much as in the posterior tooth. At the level of the lip of the alveoli, there is up to 4.5 mm of matrix between the edge of the lip and the root, giving the appearance that the teeth are "loosely" rooted. One possible explanation for the lateral position of the cheekteeth might be that they are the permanent, posterior premolars and that deciduous premolars were recently shed from their internal position (cf. Kellogg 1936, figure 38) in the life of this specimen of *L. denticrenatus*. Another relates to postulated filter feeding, and is discussed below.

The dorsal aspect of the ramus shows clearly the offset nature of the teeth. The crown of the posterior tooth is offset about 18°, that of the anterior tooth less, from the axis of the dorsal margin of the ramus. The alveoli are clearly double for the anterior tooth and bilobed at least dorsally for the posterior tooth. There is a strong wall of bone between the two alveoli in the anterior tooth, but whether this projected dorsally to fill completely the cleft between the roots is uncertain. No such bone is found in the cleft in the posterior tooth. The curvature and rise of the dorsal margin of the ramus posterior to the teeth could be interpreted as the crest ascending to the coronoid process, or it might denote the position of another tooth separated from its neighbor by a greater distance than the anterior two teeth are from each other.

Teeth:

Two cheekteeth are preserved in situ in the only two alveoli evident on the portion of the ramus preserved (Figs. 1–3, Tables 1 and 2). They are similar, but as the anterior one is damaged, the posterior tooth will be described in detail, with differences from the anterior tooth noted as appropriate. (The terms denticle and tubercle are used interchangeably.) The posterior tooth has eight major denticles splayed out anteroposteriorly on the dorsal surface of the crown. Dorsally they line up in a slightly sinuous line. There is one minor denticle anterior and one posterior to these eight, each 2–3 mm long. The denticles range from 7–13 mm in length, and are the predominant feature comprising

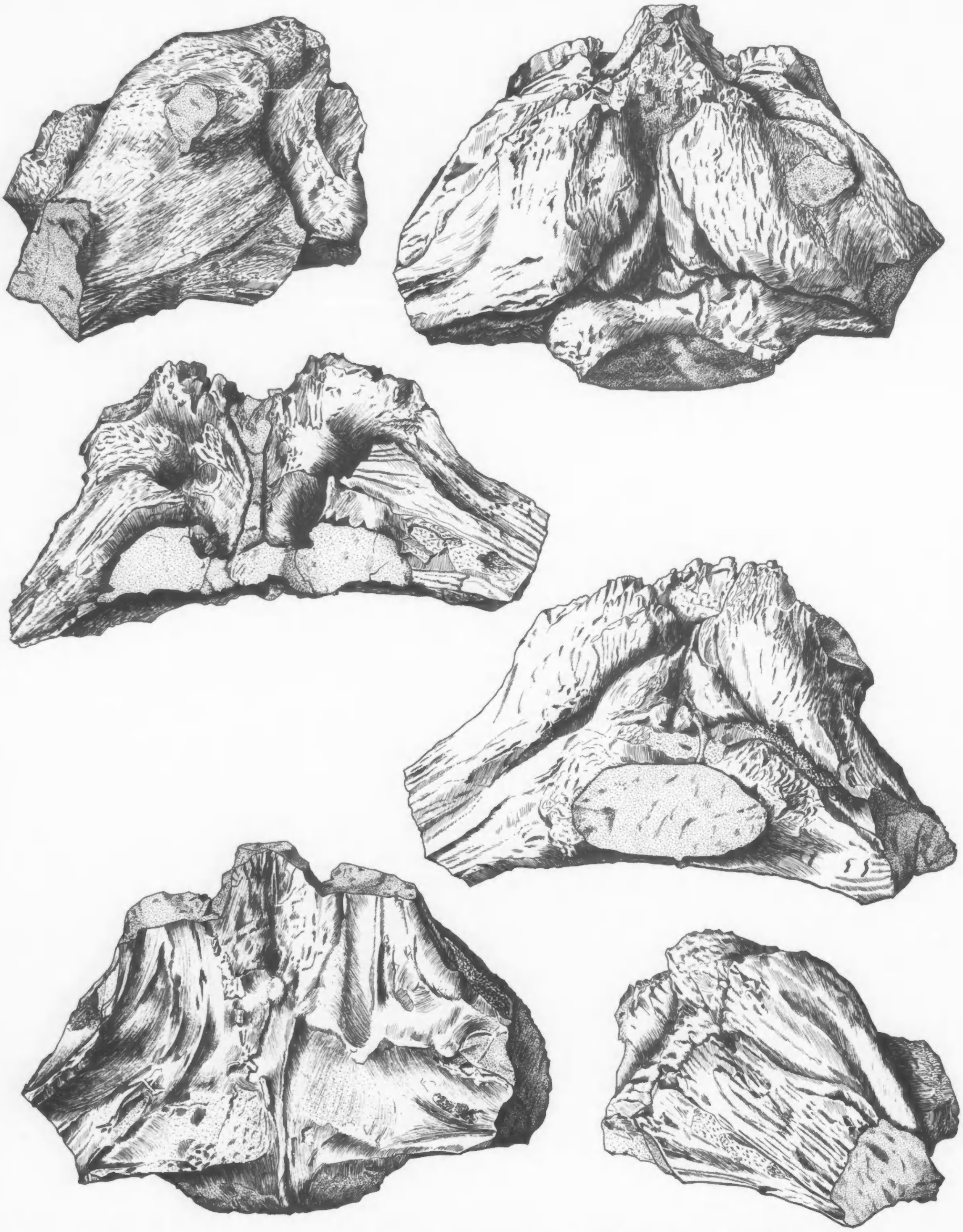

FIG. 4. *Llanocetus denticrenatus* new genus and species, natural matrix cranial endocast, referred specimen USNM 183022. Clockwise from upper left: right lateral, dorsal, posterior, left lateral, ventral, and anterior views. Specimen as preserved is 28.2 cm wide.

TABLE 1. Measurement in centimetres of dentary fragment, USNM 183022. Approximations are in parentheses.

Maximum vertical height at level of anterior tooth	12.6
Minimum vertical height between anterior and posterior teeth	12.2
Maximum vertical height at level of posterior tooth	13.4
Minimum vertical height behind posterior tooth	(13.1+)
Anterior edge of front root of anterior tooth to posterior edge of hinder root of posterior tooth	19.8
Diastema between roots of anterior and posterior teeth	10.7
Greatest lateral width of ramus	5.9

TABLE 2. Measurements in millimetres of two lower cheekteeth in situ in dentary, USNM 183022. A, anterior tooth; B, posterior tooth. Approximations are in parentheses. Approximate point to point distance between apices of denticles, sequentially, from posterior to anterior: tooth A; (8.0), (13.0), (10.0). Tooth B; 9.7, 11.4, 12.3, 11.6, 15.6, 12.2, (14.8). Maximum (vertical) length of anterior root: Tooth B; (38.8+).

	A	B
Anteroposterior diameter, at base of enamel crown	(46.3+)	41.5
Greatest anteroposterior diameter across denticles	(48.5+)	(52.0+)
Maximum transverse diameter at base of enamel crown	(14.0+)	15.8
Vertical height of enamel crown, from line between ventralmost extension of enamel anteriorly and posteriorly, to dorsalmost tip	—	42.0
Greatest height above bony alveolus, from between roots to dorsalmost tip on labial side	—	54.3
Greatest anteroposterior diameter of larger root	20.0	22.1
Greatest transverse diameter of larger root	17.6	19.5
Greatest anteroposterior length of space between roots	6.1	3.9

the crown. The open, "see-through" incisure or notch between the tubercles ranges from approximately 5–10 mm, but if one counts the 'body' of the denticle as extending in relief down onto the crown, then the 'body' of the tubercle appears to be about twice as long as wide. There is a small carina on the midline of each denticle, on either side of which the denticle is almost symmetrical.

In external view the tooth is notable for being situated not on the crest of the jaw but down on a ledge on its dorsoexternal surface. The center of the external lip of the alveolus is approximately 2.4 cm below the dorsal crest of the mandible at this level. Much more of the root shows from this side than from the internal side. The posterior-most and half of the next denticle forward lie below the level of the crest of the mandible. The denticles are splayed out, or arrayed in a broadly spaced manner, with their axes pointing from 25° off horizontal posteriorly, dorsally, and anteriorly through an arc of 110° to ca 45° off horizontal anteriorly. The central, largest denticle is oriented approximately 15° posterior of vertical. The axes of the denticles on the anterior and posterior edges of the crown curve slightly upward.

The enamel of the crown is heavily ornamented with prominent rugae oriented parallel to the axes of the denticles. The largest rugae are about eight per centimetre (measured normal to the axes of the rugae); the smallest, about 13 per centimetre. They extend from above the cingulum upward and out onto the body of each denticle but diminish short of the tip. The tips of the denticles are unworn (except for a small horizontal facet on the tip of the main denticle; Fig. 3)) and smooth except for the small carinae extending to them. The black (as preserved) enamel covering and defining the crown appears to be relatively thin and does not extend far ventrally. The cingulum on the internal surface is reduced and denoted less as a raised or thickened band of enamel than as a smooth zone of enamel into which the ornamentation does not extend. On the labial or buccal side, it is a slightly more prominent raised band which also accommodates some ornamentation and a few minute denticles. On the anterior tooth this is relatively marked, with the appearance of a double cingulum confluent posteriorly with the two posterior denticles.

The fragment of maxillary bone is badly weathered on the external surface. The piece as preserved is 9.1 cm wide, 13.5 cm long and 2.5 cm thick. It is curved such that the posterior surface of the palate must have been inflated and curved. The crown of the upper cheektooth is largely missing, but a portion with the posterior two denticles is preserved along with an epoxy cast of the natural matrix mold of a small part of the surface of the crown. The size, widespread roots, reduced cingulum, ornamentation and denticle size, shape and orientation are similar to that described above for the lower cheekteeth. There is a small denticle on the surface of the crown at the posterior end of the remnant of the cingulum. The posterior root extended approximately 6.3 cm dorsal to the enamel crown, and was approximately 1.4 cm in diameter. The unprepared fragments of a lower tooth in matrix adhering to the margin of the maxillary fragment comprise two large denticles on a fragment of a crown. The anterior denticle is situated 20 mm posterointernal to the posterior denticle of the upper tooth.

The isolated fragmentary tooth is of uncertain position. As preserved it is represented by a portion of the crown with cingulum and one denticle proximal to a complete root and a portion of the other root. The intact root is 4.4 cm long and 15.5 mm in greatest transverse width. The portion of the crown preserved is 12.8 mm in width. The cingulum is more pronounced, there may have been fewer denticles on a smaller crown, and the roots are separate but closer together than in the cheekteeth described above. It also differs in having a distinct shoulder or protuberance on the root where it is separated from the end of the crown by a deep groove. All the teeth have roots with the inflation or greatest diameter at the proximal end, nearest the crown.

Description of referred specimen — Preservation, size, and overall form:

The fossil endocast (USNM 183022: Figs. 1,4) is comprised of a natural cast of fine-grained, dark gray sandstone. It shows much large-scale surface topography and fine-scale positive casts of vessels and canals. On the surface there are remnants of bone and/or matrix weathered to an ivory colour scattered in concave endocast features on the dorsal midline, right side and anterior end. Thin, irregular sheets of remnant bone on the ventral surface are dark gray, slightly darker than the rock comprising the cast. Thus the endocast appears to represent nearly all of the endocranial space of the skull. It shows prominences and bulges on the dorsal surface that may or may not reflect brain structure. Since there are deep sulci separating these prominences on each side, and separating right and left prominences, they likely do reflect at least in a general way some aspects of the structure of the brain in this species.

The endocast is broken off in a neat transverse plane at the foramen magnum. The large, posteroventrolateral extensions of the cast are both broken off. The left one is a clean break normal to the axis of the extension. The right one is broken off shorter and in a more dorsally oblique plane, and it is more irregular and weathered. On the right dorsolateral surface, there is a teardrop-shaped surface denoting a piece missing, but this is only a thin piece, judging from the undamaged surface at this location on the left side. The anterior surface of the endocast is largely intact, with most lobulations or other convex features 'feathering-out' or terminating anteriorly into rugose-surfaced bony walls. The exceptions are a transverse break across the anteroventral margin of the cast at about the same level on both sides, and approximately 2–3 cm anterior of this plane, a transverse break at the midline along a vertically oriented structure. All these broken surfaces are indicated by fine stippling in the figures (Fig. 4).

The size of the endocast approximates that of a living mysticete about the size of an adult *B. acutorostrata*. The fossil as preserved is 28.2 cm wide, 19.3 cm in maximum length (approximately 18.0 cm in standard length along axis of brainstem) and approximately 13.0 cm in greatest height. The volume of the entire endocast as preserved is 2525 cm^3, substantially greater than the volume of the brain in life.

The overall form of the endocast is triangular in dorsal view, pyramidal with a broad and deep sulcus at the top in posterior view and a truncated triangle in ventral view. The ventral surface is broadly concave by 2.0 cm or more. The posterior and dorsal surfaces show lateral projections, deeply defined dorsal lobes or hemispheres and a 5.0 × 10.2 cm ovoidal posterior area well defined by a break surface that represents a cross section of the foramen magnum. The anterior view shows the transverse break of irregular surface, shaped like an upside-down T, with most other structures and surfaces fairing from this posteriorly in sweeping curves but with rugose to striated textures. Lateral views emphasize the horizontal axis of the brain with a large structure placed across it obliquely, dorsomedial to ventrolaterally. The area above the foramen magnum, where the bony supraoccipital shield lay, slopes markedly forward at an angle of approximately 40° up from the base of the endocast. It is an area of deep relief and structural complexity, showing four pairs of globose, vascularized structures.

Caution on structures represented by fossil endocast:

There is a well-founded literature on the cetacean brain, with useful reviews of 19th century knowledge by Guldberg (1885) and more recent overviews by Breathnach (1960); Jansen and Jansen (1969); and Morgane and Jacobs (1972). Good, well-illustrated monographs describing the brain of mysticete species include those by Beauregard (1883); Friant (1958); Langworthy (1935, 1938); and Wilson (1933). There is also a substantial literature on cetacean endocasts, dating well back into the 19th century. In the early 20th century, some interest focused on the endocasts of Archaeoceti and other cetaceans for clues to their mammalian relationships and habits. Studies of Smith (1903); Stromer (1908); Dart (1923); and Edinger (1955) were followed by those of paleontologists (Kellogg 1928; 1936; Flynn 1948), all of whom inferred that endocasts of the cranial cavity of archaeocetes reflected the true form of an archaeocete brain that was unique among mammals.

Marples (1949) suggested that the supposed immense and unique cerebellum inferred from archaeocete endocasts resulted from a misinterpretation of a large vascular plexus. Such vascular networks, or retia mirabilia of Owen, had been found in *Balaenoptera physalus* extending "into the skull and through the foramen magnum and embracing the back of the brain" (Ommanney 1932, p. 348). Walmsley (1938) gave illustrations of the blood vascular plexus in the neck, beneath the basicranium and within the endocranial cavity in living mysticetes. Breathnach (1955a) compared endocranial casts (e.g. Gervais 1871) made from the cranial cavity of skulls of extant cetaeans, and the preserved brains of the same species. He concluded that in the smaller odontocetes the endocast gives a reasonable picture of the form and proportions of the brain, but with some distortion of the cerebellar region. In mysticetes, the presence of extensive intra-cranial retia makes the endocast "little less than a poor and misleading caricature." Breathnach (1955a) accepted Marples' (1949) interpretations and concluded that most previous findings about the enormous cerebellum in archaeocetes were incorrect. He also considered as suspect Dart's (1923) view of an enormous trigeminal hypertrophy in archaeocetes, pointing to the same error in misinterpretation of enveloping retial tissue.

Description and interpretation of structures on endocast:

The above cautions indicate how little a natural endocast from a fossil cetacean can be expected to show in the way of evidence of brain tissues. Nevertheless I mention some prominent structures, describe them and indicate the most likely and some alternative interpretations of what they might represent. In considering the endocranial morphology of the mysticete skull, I have referred to papers by Beneden and Gervais (1880); Burlet (1914); Fraser and Purves (1960); Honingmann (1917); Miller (1923); Ridewood (1922); and Schulte (1916). The endocast shows a wide combination of features, with a ventral surface relatively flat and featureless like that of living mysticetes (Gervais 1871, pls. 8–10) but with a topographically varied and complex dorsal surface with well-defined vascularized lobes and deep fissures. The major features are illustrated in Figs. 1 and 4, and some mention is made of the terms used by Dart (1923), whether correct or not.

The identification of the gross features of the dorsal surface of the endocast is difficult, and it affects the comments offered below. Basic identification of the three paired, globose structures of successively smaller size from front to rear is the problem. For purposes of this discussion, I term them from front to rear, A, B, and C. If these were exceptionally preserved soft tissues insulated from the braincase wall by now-missing retial/vascular tissue, small bone fragments would not be scattered on the surface and in sulci and fissures. Thus the endocast represents the true configuration of the endocranial cavity dorsally (and elsewhere). That being the case, I follow Breathnach (1955a) in assuming that most or all brain surfaces are covered by retial/vascular tissues. Whether some of these are sufficiently large to form globose structures in and of themselves, such as B and C, is an uncertain point. The simplest interpretation (Interpretation I) of A, B, C is: A is a reflection of the cerebrum, covered by vascular and other tissues with a paired retial mass on the posterodorsal surface of the cerebrum. B and C are reflections of the cerebellum, covered by vascular and other tissues. The problem with this interpretation is that B and C are bilateral structures separated on the midline by deep, vertical fissures, unlike the typical mammalian cerebellum. (Interpretations of B and C as substructures of the cerebellum are unlikely, but not impossible. The mysticete cerebellum appears to be laterally enlarged (cf. Jansen 1953; Breathnach 1955b). The cerebellum of *B. acutorostrata* is grossly bilateral (cf. Jansen and Jansen 1969, figure 5).

Alternative Interpretation II of A, B, and C is as in Interpretation I but omitting the most posterior, small, bilobed structure (C) from consideration due to its being part of the vascular system. Then A might be the cerebrum and B the cerebellum. This still begs the issue of the species having a bipartite cerebellum, which would be exceptional for a mammal.

Alternative Interpretation III is that A is mainly retial/vascular tissue, B a small cerebrum and C an even smaller cerebellum. Problems with this interpretation include the paired nature of C and the special case of calling A such a tissue mass without also calling B and C similar tissue masses.

Alternative Interpretation IV is based on comparison with other unknown structures in the endocasts of e.g. *Dorudon*(?) *sensitivus, D.*(?) *elliotsmithi, D. intermedius*, and *Prozeuglodon isis* (Dart's *P. atrox*) as described and illustrated by Dart (1923). The enlarged, lobulated structures at the posterodorsal surface and margin of the endocast that Dart interpreted as cerebellar structures (paraflocculus, lobus medius cerebelli), and which are more likely retial tissue (Breathnach, 1955a), might be the same tissue masses with the lateral one ("paraflocculus") extended anterodorsomedially up onto the "cerebrum" (A), and the more medial ones ("lobus medius cerebelli") represented by, from front to back, B, C and structures atop the brainstem.

Alternative Interpretation V is not mutually exclusive with IV. It is that the largest dorsoanterior structure, A, is part of an extension of blood vascular and related tissue from the middle ear system relevant to pressure equilibration, oxygenation, and thermoregulation during diving. In this case, B would be or reflect the cerebrum and C would be reflections of cerebellum.

The most likely case is Interpretation I. It is the simplest, and can also be related to cranial telescoping. The retial mass in archaeocetes is at the level of the jugular leash, atop the cerebellar portion of the brain. The supraoccipital of archaeocetes is vertical. In the telescoped skull of mysticetes (sensu lato, below) the supraoccipital is slanted forward over a foreshortened braincase. Thus in *L. denticrenatus* with a sloping supraoccipital, the retial mass is displaced anteriorly over the cerebral portion of the brain. Structures posterior to this are reflections of cerebellar morphology.

I do not know which interpretation is correct. Better interpretations must await the discovery, preparation, and description of cranial material in which endocast impressions can be matched with appropriate cranial landmarks.

Some structures can be identified with a little more certainty. What Dart (1923) called the olfactory peduncle is an anterior protuberance on the midline of the endocast, extending 8.8 cm vertically. Dorsally it is a tripartite structure 2.4 cm across at its widest, below this it is as little as 0.5 cm wide, and ventrally it is laterally expanded so as to be confluent laterally with what Dart called in this region the Gasserian (or tirgeminal) ganglion. It is broken cleanly off, so its anterior shape and extent are unknown. Alternative explanations are that this structure is the anterior portion of retial or vascular tissues around the anterior end of the cerebrum, or part of the turbinals or cribriform plate. The protuberance is on the dorsal midline, median and unpaired, and occupies the site of what in archaeocetes is termed the olfactory peduncle. The irregularities filled with thin, curved bone fragments in its anterior surface are of the appropriate shape and location to be some of the scroll-shaped ethmoturbinal bones. Thus this structure might comprise the entire olfactory tract and bulb. If so the dorsal position is like extant mysticetes and the foreshortening is unlike archaeocetes.

The sagittal sinus of Dart (1923) is a depression in the dorsal surface topography, the surface of which is covered with fragmentary and interdigitated bone remnants. The depression is at least 3.2 cm deep, below the apices of the prominent dorsal-most bosses (A). It is triangular in shape, with one apex pointed posteriorly on the midline. Since bone fragments cover this region, I am not convinced that it represents anything other than where the dorsoanterior wall of the cranial cavity lay.

The median tentorial depression of Dart is where the bony tentorium would lie. The fissure or cleft between the dorsal paired structures is deep and V-shaped. There are remnants of bone and/or matrix in the cleft.

The cerebral hemisphere of Dart is a paired structure (A, see alternative interpretations, above), comprised of a flattened hemisphere, irregular in shape but with an anterodorsal apex. The apex is pitted and rugose where it interfaces with longitudinal striae and rugae in the braincase wall. Laterally and posteriorly the surface texture is comprised of smoother rugae and pits. It seems logical that this is a reflection of the retial/vascular tissue-covered cerebral hemisphere. It is a paired, anterodorsal, globose structure located in the normal position of the mammalian cerebrum. (If so, the longitudinal cerebral fissure was a major space 1.4 cm or more in width.) The anteroexternal margin of this anterodorsal-most structure (A) shows positive casts of blood vessels and possibly bony trabecular structures, and it may represent a vascular/retial tissue mass. This appears to be a flattened sheet of tissue. The entire mass appears to have been fed from the posteroexternal corner by four or more vessels of 1.5 – 2.0 mm diameter. The right tissue mass measures approximately 10.7 × 4.4 cm and was at least 5 mm thick. The posterointernal surface of the tissue mass also shows convolutions and positive casts of vessels. My identification of this structure is based upon the general comments of Breathnach (1955a).

The paired structures (B) posteroventral to the largest dorsalmost lobes (A) are comprised of bilaterally paired lobes. (Since they do not, taken together, represent a hemisphere, that term is not used here.) As exposed, these are 9.5 cm in maximum lateral width, 6.3 cm in maximum length. There are positive casts of blood vessels distributed over the surface of each. Does this indicate that they themselves are not retial tissue, but a structure supplied by blood vessels? Posteriorly they are splayed apart with a space of up to 3 cm between them, but anteriorly they are adpressed over at least one half their exposed length. The plane of their greatest width lies tilted approximately 40° ventroexternally from the axis of the endocast. The simplest anatomical identification of these paired lobes is that they represent tissues overlying the cerebellum (but see alternative interpretations, above).

The smallest, posterior structures on the dorsal surface consisting of small, bilaterally paired lobes or protuberances (C) are small, slightly irregular in orientation and generally mammiform in shape. They protrude approximately 7 mm from adjacent pits and fissures and are about 3.0 cm in combined width. In dorsal view, they are exposed for a length of about 2.0 cm. They are closely adjacent to what are probably positive casts of blood sinus channels in the surface of the occiput. The simplest anatomical identification of these structures is that they are part of the cerebellum. However, as pointed out above under other interpretations, they are paired and not median or tripartite. They could even be paired occipital sinuses associated with the blood vascular system.

The occipital sinus is probably represented by a positive cast of a channel or canal in the bone of the supraoccipital and is preserved on the right side only due to some bone, being left in place. It runs from above the foramen magnum directly anterior and dorsal to the right half of structure C. If it is not part of the occipital sinus system, it might be associated with a retial system.

The tissue surrounding the nerve cord at the level of the foramen magnum was called the "medulla oblongata" in archaeocete endocranial casts by Dart (1923). Breathnach (1955a) noted that Dart had concluded that this portion of the brain was widened due to hypertrophy of the tuberculum quinti (spinal nucleus and tract of the trigeminal).

There is a vascularized tissue mass just inside the foramen on the dorsolateral surface of the brainstem. This is a relatively flat, teardrop-shaped, paired tissue mass, the pair being separated by a distance of ca 4.5 cm dorsally. The right tissue mass is at least 8.8 cm in lateral dimension along its longest axis and is exposed at least 4.0 cm in its lesser dimension. The surface is rugose and appears to consist of highly vascularized structures. This vascularized tissue mass lies in an anatomical position surficial to the medulla oblongata, occupied by the choroid plexus in most mammals. If this is not the posterior choroid plexus, perhaps it is a retial mass.

The transverse section of the foramen magnum is 5.0 cm high and 10.0 cm wide. Positive impressions of vascular tissues occur on the external apices of this cast of the foramen magnum. The dorsal and ventral margins and adjacent surfaces are smoother. The cross-sectional shape of the foramen magnum is relatively wider, dorsoventrally flatter, and with more distinct lateral apices than in archaeocetes (Dart 1923) or living mysticetes (Gervais 1871).

The jugular leash of Dart is represented on the endocast by a ridge, or a positive impression of a groove or channel in bone, approximately 1.0 cm or less in caliber. It runs along the posterodorsointernal margin of the Masses annexes of Gervais (1871), or acoustic nerve of Dart. On the right side it passes across or from the dorsoanterior edge of the 'posterior choroid plexus', whereas on the left it appears to run anterior to it. Dart named the endocast impression of the jugular vein and associated nerve structure the "jugular leash."

The Masses annexes of Gervais are massive structures, comprising approximately one-third of the bulk of the endocast. It runs on each side posteroventrolaterally from the anterior region of the endocast, down to and through the cranial fissure or cranial hiatus to the middle ear region. In the endocast it comprises retial and vascular tissue surrounding the cerebellum and accompanying cranial nerves out of the skull. Dart called this endocast structure the "acoustic nerve."

On the anteroventrolateral margin of the endocast is a large, concavely arcuate structure called the Gasserian ganglion or trigeminal ganglion and nerve by Dart in archaeocete endocasts, but retial tissue accompanying the nerve and its branches by Breathnach. Its shape in this endocast resembles closely that illustrated by Gervais (1871) in plaster endocasts taken from skulls of extant species of mysticetes. As seen in transverse section in an anterior view of the endocast, the irregular cross section of this mass of tissue does not allow the identification of the precise position of the maxillary nerve or opthalmic nerve as Dart attempted to do.

A small protuberance on the ventral surface of the endocast represents a fossa in the basicranial floor for the hypophysis. This protuberance on the endocast is approximately 1.7 cm long and 1.1 cm wide. It is raised from the endocast surface approximately 0.5 cm in relief. There is no well defined *sella turcica* evident in the specimen as preserved, although there was a bony spine or spur anterior to the fossa, perhaps the posterior end of a bony ridge.

On the ventral surface of the endocast, running forward along the ventral midline is a cast 5.1 mm in diameter of a blood vascular channel entering the braincase on the midline inside the foramen magnum below the nerve cord. The channel may have accommodated the basilar artery or the entrance of the cranial rete mirabile from the spinal rete. If the former, then the paired vessels into which it diverges could be the posterior cerebral arteries.

Discussion

Feeding apparatus:

The widely-spaced double roots of the teeth, the large and widely-spaced unworn tubercles on the anterior and posterior edges of the crowns splayed out so far as to point directly posteriorly, the ornamentation on the crowns comprised of rugae, the wide spacing between cheekteeth, and the evidence for a gum line all indicate that *L. denticrenatus* did not use the teeth in grasping, piercing, and retaining struggling nektonic prey such as squid or fish. It was likely mainly a filter feeder, possibly feeding like the living Crabeater seal. *Lobodon carcinophagus*.

The Crabeater seal's cheekteeth have similar, unworn, ornamented tubercles on the anterior and posterior edges of the crown, deeply divided and widely separated from one another (figures in Scheffer 1964; Kooyman 1981). The posterior upper and lower cheekteeth are adjacent to posterior upper and lower bony protuberances (Gray 1874, pl.9) which, when covered with soft tissues in life, must effectively close the gap behind the posterior teeth and may augment the straining function of the last cheekteeth (King 1961). The Crabeater is a specialist feeding almost exclusively on krill or euphausiids (Bertram 1940; Laws 1977) as does the largest of animals, the Blue whale *Balaenoptera musculus*. Some consider the crabeater seal one of the most abundant wild large mammals in the world (Laws 1983). While it is clear from examination of stomach contents that they are nearly stenophagous on euphausiids, their feeding behaviour has not been closely observed underwater. Racovitza (1900) and Barrett-Hamilton (1901) commented on their filter feeding mode and compared it with that of mysticetes. There are few observations (Bertram 1940; Kooyman 1981), and the precise details of how the Crabeater seal filter feeds are not well documented.

I speculate that *L. denticrenatus* fed on euphausiids and/or their early Tertiary trophic equivalent (which might have been a mix of invertebrates and fish or even small vertebrates exclusively). Euphausiids essentially have no fossil record. Fish would have been the energetically more desirable prey. *L. denticrenatus* might have fed on schooling fish of the order of 10–20 cm in length or larger, as does the fin whale and the feeding generalist among the extant mysticetes, the humpback whale, *Megaptera novaeangliae*. The tubercles in the two cheekteeth are equidistantly spaced with similar-sized, V-shaped notches between them. The notches are all approximately 1 to 5 mm or more wide from the apex to the open bottom of the V. Larger euphausiids are on the order of 2.0 cm or longer in size. Thus the scale of the hypothetical fossil filter matches the scale of the organism on which a living parallel, the Crabeater seal, is adapted to feed. Living mysticetes likewise feed on prey about this size, with exceptions in copepod (smaller) and fish (larger) feeders (Nemoto 1959; Mitchell 1975).

The longitudinal spacing of the teeth is a problem for my interpretation of *L. denticrenatus* as a filter feeder as it might require the presence of special structures such as enhanced gum, lip, or other soft tissues for which there is no fossil evidence. Some teeth were apparently spaced out on the order of 15.5 cm center to center or less, such that upper teeth with crowns approximately 5 cm in length like the lower cheekteeth would not have fully filled the intervening space. Two 5-cm long spaces might have been filled or blocked with lip, gum, or palate soft tissues, analogous to the bony posterior flanges blocking off a similar space at the rear of the Crabeater's tooth row (King 1961). This would have been efficient if the prey were larger items such as small fish or larger invertebrates a few centimetres or more in length. The tooth notches, even if closed off only partly by lip flaps, the opposing palate or the tongue, would have served as a sieve for the escape of water from the mouth. (The most efficient position for rear teeth functioning in this manner would have been outside the crest ascending to the coronoid process, near the corner of the mouth which functioned as an excurrent channel, rather than inside or on the crest as in most archaeocetes where their function was to chew and retain food for passage down the gullet.) The efficient function of the feeding mechanism might not have been entirely dependent upon relatively static soft or hard tissue structures — the lip, tongue, and other oral structures probably played active and complex roles in filter feeding. It is precisely the selection pressures acting on the soft tissues required in this speculative filter-feeding mechanism that would have comprised the substrate for variation and selection resulting in the evolution of baleen.

The 'dental gutter' (Schulte 1916, p.483, pl.57), 'alveolar sulcus' (Reinhardt 1869, p. 1, figure 2) or 'dental groove' (Ridewood 1922) containing the vestiges of teeth in mysticetes runs posteriorly along the dorsal margin of the dentary and ends where the margin ascends as a slight crest to the coronoid process (Beneden and Gervais 1880, Atlas, pls.1–2, figure 8). The posterior end of the tooth row in archaeocetes is on the dorsal margin of the ramus even where the crest ascends to the coronoid process, and ends on the crest in many species or slightly medial (lingual) of the crest in *P. lugardi* and some other species. The posterior end of the dentary tooth row of *L. denticrenatus* occurs markedly off the dorsal margin, external (labial) of it if the above identification of the holotype dentary fragment as from the right side is correct. Thus *L. denticrenatus* also differs from archaeocetes and mysticetes in this offset of at least the posterior portion of the tooth row from the dorsal margin of the mandibular ramus. The lineal axis of the mental foramina for exit of branches of the interior dental artery, associated with the line of the lower lip, is similarly offset posteroexternally in at least one extant species (Beddard 1901, pl. 8), possibly reflecting the position of the angle of the gape in its function as an excurrent flow channel.

Endocast:

The endocast represents a cetacean cranial cavity unlike that of any described archaeocete. It differs from those known (Dart 1923) in that in lateral view, the area above the foramen magnum and cerebellum shows none of the structures called the paraflocculus and lobus medius cerebelli by Dart and retial tissue by Breathnach. It also differs in that this area of the bony supraoccipital shield may slope anteriorly at a much greater angle (ca 45°) than in any archaeocete (where it is nearly vertical, ca 90°). In extant mysticetes the supraoccipital shield is strongly angled anteriorly (Miller 1923). The vertical extent of the cast associated with the olfactory peduncle does not resemble the morphology in archaeocetes. The topographical complexity of the structures on the dorsal surface of the endocast and the degree to which they were surrounded by a braincase with a large tentorium and other structural rugae and struts differ substantially from archaeocetes, and all other described cetaceans and other aquatic mammals. This includes endocasts of mysticetes (Gervais 1871), endocasts of odontocetes such as *Prosqualodon davidi* (Dart 1923), and *Cyrtodelphis sulcatus* (Dal Piaz 1905), the brain of odontocetes such as *Delphinapterus leucas* (Pilleri 1963) and *Platanista gangetica* (Pilleri 1972), and the brain or endocast of pinnipeds (Jelgersma 1934), sirenians (Edinger 1939) and hippopotamuses (Friant 1940). The ventral surface bears a marked resemblance to that of endocranial casts of living mysticetes (Gervais 1871), especially in the size and shape of the retial tissues accompanying cranial nerves and other structures through the skull (Masses annexes of Gervais), in the relative size and placement of the hypophyseal fossa (and absence of a typical *sella turcica*) and in the shape of the anteroventroexternal margin of the endocast curving from the Masses annexes to the area of the tissue around the maxillary nerve of the trigeminal. The endocast would have to be identified as that from an extant mysticete if only its ventral morphology were known.

The brain of living mysticetes is notable for its large absolute size and large, cerebral hemispheres. The brain of *L. denticrenatus* would have had relatively smaller cerebral hemispheres no matter which alternative interpretation is made of dorsal structures on the endocast. The extensive vascularization resulting in intracranial rete has been well described in living mysticetes by Walmsley (1938, figures 25,27). He found that the extensive cranial rete mirabile (= rete arteria carotis) was directly continuous with the spinal rete mirabile through the foramen magnum. It apparently enters the cranium ventral to the spinal cord. Inside the cranium the left and right vessels curve around the base and side walls and meet atop the body of the basisphenoid where they form a vascular mass surrounding the hypophysis. From this mass, anterior and posterior cerebral arteries arise "in the same way as the roots of a tree" (1938, p.166). There is no direct evidence that the cranial rete on the floor of the braincase of *L. denticrenatus* differed substantially from that of living mysticetes, but since some of the blood vascular routes were incised into bone the ventral cranial rete may not have been as well developed.

The presence of probable turbinal bones allies *L. denticrenatus* with Mysticeti where they are characteristically present, rather than with extant Odontoceti where they are absent (Weber 1928, Figure 229; Oelschlaeger 1987, p. 672).

The foreshortening of the endocranial cavity apparently resulting from some degree of telescoping, the mysticete-like Masses annexes of Gervais and jugular leash of Dart, the U-shaped 'olfactory peduncle', the hypertrophy of apparent retial tissue on the dorsal surface of the brain, the paired structures dorsal to the brain stem area (if not also retial tissue) and other characters described above represent a strange combination of features. Some are found in archaeocetes, others in mysticetes, and yet others were heretofore unknown in Cetacea. Taken together they indicate that a radical new kind of cetacean is represented by the fossil. Full understanding of the identity and significance of some of these structures will have to await the further description of endocasts from other Late Eocene and Oligocene species.

Comparisons:

Other fossils referable to archaeocetes were earlier collected on Seymour Island (Wiman 1905; Abel 1919; Kellogg 1936). Woodburne and Zinsmeister (1984) note that whale remains occur sporadically throughout the rock unit, and that the material Wiman (1905) described "was collected from a single locality near the top of the La Meseta Formation. During the 1981–82 season, additional whale remains tentatively referable to *Basiliosaurus* (sic) were found at a number of horizons throughout the La Meseta Formation, including RV-8200, with *Antarctodolops* new genus." Thus the Wiman specimen might have been collected near the type locality of *L. denticrenatus*. Other cetacean fossils also have been collected recently near the same locality (Anon. 1987)

Some aspects of the origins of Archaeoceti have been documented (Kellogg 1936; Sahni and Mishra 1971; Gingerich et al. 1983; Oelschlaeger 1987) as have been the characteristics of typical archaeocetes (Kellogg 1936; Barnes and Mitchell 1978) and variants (Delfortrie 1873; Hector 1881; Winge 1921; Kellogg 1936; Kumar and Sahni 1986). A number of these and other species have been proposed as mysticete ancestors, and most workers tend to discuss these for convenience in terms of their generic-level characters. *Patriocetus* and a somewhat similar genus *Agriocetus* are now regarded as odontocetes related to squalodontoids (Whitmore and Sanders 1976). *Archaeodelphis* was thought by Kellogg (1928) to be a plausible mysticete ancestor, but it is much younger than known true mysticetes. *Ferecetotherium* and *Mirocetus* (Mchedlidze 1970, 1976, 1984) may stand in the archaeocete-mysticete evolutionary line (Whitmore and Sanders 1976), but this has not been convincingly argued. I agree with Barnes (1985) that *Ferecetotherium kellogg*; shows physeterioid features. The teeth and dentary of *F. kelloggi* are unlike those of *L. denticrenatus*. *Mirocetus* was placed in the Patriocetidae by Mchedlidze (1970), and *Patriocetus* is regarded as an odontocete (Miller 1923; Rothausen 1968).

Recently Fordyce (1981, 1982a,b, 1984, 1988) has suggested that *Mammalodon colliveri* (Pritchard 1939) represents an intermediate stage in the transition from archaeocetes to mysticetes. The skull shows archaeocete-like features (Fordyce 1984) but has a strong heterodont dentition with closely appressed, incipiently double-rooted teeth having strong cingulae, and the dentary contains a large, well-excavated mandibular canal like that of odontocetes (pers. observ.). The coronoid process is large and well developed, with an irregular internal surface. The extreme wear of the crowns of the six lower cheekteeth along one horizontal plane indicates that the dentition occluded crown to crown. Odontocete top predators such as the killer whale, *Orcinus orca*, sometimes show this condition in aged individuals, those pursuing atypical uses of teeth, or with cranial abnormalities resulting in a misalignment of the dentary (cf. Mitchell and Baker 1981, p. 147, figure 3). It might also result from a feeding behavior in which abrasive material such as benthos is mouthed or taken into the oral cavity when capturing prey, or where the prey has hard parts. The posterior slant of the lower cheekteeth is reminiscent of *Patriocetus erhlichi*. These features of *M. colliveri* preclude it from further consideration as a close relative of *L. denticrenatus*.

The earliest apparently true mysticetes, *Mauicetus* spp. (Benham 1937a, 1939, 1942; Marples 1956) from New Zealand, are mid to Late Oligocene in age and "adult individuals of *Mauicetus* were toothless, almost certainly possessed baleen, and had a true, primitively telescoped, mysticete skull" (Fordyce 1977a) with a long intertemporal area and an anteriorly thrusted triangular supraoccipital. Another Late Oligocene true mysticete, *Cetotheriopsis tobieni* (Rothausen 1971), appears to show more derived characters than species of *Mauicetus*. Subsequent mysticete evolution was summarized by Kellogg (1928).

I note that the number and the linear arrangement of mental foramina for exit of the inferior dental arteries show a consistent and similar arrangement in Archaeoceti and Mysticeti. Examples include: seven foramina from near the level of I_3 posterior to the level of Pm_4 in a protocetid (Halstead and Middleton 1976, figure 4); seven foramina on one side and up to nine on the right side between I_1 and Pm_4 of a basilosaurine (Kellogg 1936, p. 29); and at least seven major plus five minor foramina from I_1 to M_1 in a dorudontine (Kellogg 1936, figure 31a). The spacing of the major foramina appears to approximate the spacing of teeth. In mysticetes there is a similar pattern of foramina (not to be confused with the often more numerous small nutrient foramina in the remnants of the dental groove): six to seven foramina in two specimens of cetotheres (Kellogg 1968, figures 40, 49); seven foramina in a neobalaenid specimen (Beddard 1901, pls. 8,9); approximately seven to eight or more in two eschrichtiid specimens (Cederlund 1940, p.271; Andrews 1914, pl. 27); three to seven in five balaenid specimens (Beneden and Gervais 1880, Atlas, pls. 1–6); and seven to nine in four balaenopterid specimens (Beneden and Gervais 1880, Atlas, pls. 9–11, 14–15). The consistency is striking and may be related to the number of inferior dental arteries running through the mandibular canal. Walmsley (1938) studied fetal and adult material of the fin whale, *Balaenoptera physalus*, and found that as one of the branches of the maxillary artery, "seven inferior dental arteries arise close together in linear formation. They pass ventrally to the mandibular foramen and traverse the canal in which they give off branches to the labium. Each artery is surrounded by several veins which open into the pterygoid plexus." In one adult he cut the mandibular ramus at midlength and found 10 arteries and accompanying veins in the canal (1938, figure 35). The number and distribution of mental foramina may be synapomorphic for Cetacea in phylogenetic terminology (Wiley 1981) since essentially the same pattern is found in primitive Odontoceti (but not in highly derived forms like Delphinidae and some other lineages). This may be further evidence of cetacean monophyly.

The morphological hiatus between appropriate archaeocete ancestors (Dorudontinae, Barnes and Mitchell 1978) and mysticetes with a telescoped skull, wide and flat rostrum and reduced dentition now is thought to be occupied by the relict form *Aetiocetus* (Emlong 1966; Van Valen 1968) and a near relative *Chonecetus* (Russell 1968; Barnes 1987, 1989). Both are Middle and Late Oligocene primitive cetaceans and although they have the full complement of mammalian teeth, Barnes (1987, 1989) identified them as mysticetes, not archaeocetes, because they possess derived mysticete cranial features even though no known aetiocetid species was a direct ancestor of baleen-bearing mysticetes. These derived characters include a lateral process of the maxilla anterior to the orbit, "a ligamental mandibular symphysis and associated groove", and the size and distribution of the pterygoid sinuses (Barnes 1989).

The groove on the internal surface near the distal end of the dentary in extant and some extinct mysticetes occurs variously as a thin, suture-like crack to a major sulcus with an overhanging dorsal lip but open ventrally. It turns up gently to sharply just posterior to the distal end. It is pronounced in balaenopterines, but not in balaenids. I confirmed the presence of the sulcus in a dissection of the jaw of a 295 cm long fetus of *B. physalus* (EDM specimen WF-2). The sulcus is 10.5 mm dorsoventrally wide approximately 140 mm posterior of the tip of the dentary. The dentary at that point is 43.4 mm deep and 28.2 mm wide. The sulcus is occupied by Meckel's cartilage, which at that point is 5.6 mm transversely wide and partly fused with the bony tissue of the dentary. Anteriorly it tapers to a thin, free tip embedded in the ligamentous tissue of the symphysis. The relationship of the cartilage to the dentary bone is like that illustrated by Ridewood (1922, figure 1). The sulcus appears to be related to the origin of the mylohyoid muscle (Schulte 1916, pl. 57, figure 2), and True (1912) equated the two features in evaluating fossil dentary bones of mysticetes. The anatomical and functional relationships between Meckel's cartilage, the fibrocartilage skeleton of the ventral pouch (Pivorunas 1977), and the fibrocartilage connection of the symphysis (Lillie 1915, Pivorunas 1977) of balaenopterines is uncertain.

My initial concept of Mysticeti was of a clade defined by the presence of baleen and the loss of teeth in adults. The feeding mechanism of living mysticetes is a complex interplay of morphology and specific behaviour (Fraser 1952; Pivorunas 1977, 1979; Lambertsen 1983). Structures such as the enlarged fibroelastic connection of the mandibular condyle to the glenoid fossa and the fibrocartilage skeleton of the ventral pouch do not leave unequivocal signs of their presence in the fossil record. For example, various authors have cited the broad vascular canals (Walmsley 1938, p. 142) on the palate of balaenopterids, cetotheres, and other taxa as evidence for the presence of baleen. (The surficial canalization appears to be slightly different in eschrichtiids and not as abundant in balaenids.) Blood vascularization in the palate and gum of these mysticetes may have a primary function in the rapid growth of baleen in utero, but it might also function in thermoregulation in the adult. Ontogenetically baleen arises as a horn tube in the epidermis external to the dermal papillae of the basal plate on the palate (Fraser 1952; Van Utrecht 1966). (All other parts of the baleen plate, including compacting horn between the horn tubes, the covering layer of horn and the intermediate substance of softer horn between plates arise from the epidermis external to the basal plate.) The long tapering papillae extend far down into the horny tubes (Tullberg 1883), which are anatomically hollow and open to the environment at the frayed distal and where the free tubules are best termed fringes (Pivorunas 1976). Cross sections of the papillae show that they contain an artery surrounded by veins like the counter-current heat exchange structure in appendages (Van Utrecht 1958). Gum tissue also contains the same structure. Thus these tissues of the oral cavity may play an important role in baleen whale thermoregulation (Van Utrecht 1958). Epidermal papillae may have evolved in part due to thermoregulatory selection pressures. Investment of papillae with horny tissue may have been the result of mechanical, water flow or other selection pressures such as aiding the concentration of retained prey into a condensed bolus for swallowing. Baleen is like hair, horns and digital tips in other mammals and, like horns, it may have evolved more than once, perhaps in some form different from that in extant whales. The blood vascularization of the palate may not be an exclusive indicator of the presence of baleen in fossil whales. More precise, multiple criteria need to be established for the interpretation of baleen and other "soft tissue" structures in some important extinct species.

Comparison of the teeth of the new species *L. denticrenatus* with superficially similar teeth from the holotype of *Kekenodon onamata* Hector 1881, and the single, holotype tooth of *Phococetus vasconum* (Delfortrie 1873) shows some similarities. Various fragments referred to the skull and skeleton of *Kekenodon onamata* have been found (McKay 1882a,b; Benham 1937b), but the teeth and periotic of the holotype are best described (Hector 1881; McKay 1882a,b; Kellogg 1936; Wilson 1973; Fordyce 1977b) and show distinctive characteristics. The teeth of all three species are large, have high crowns with deeply divided accessory denticles or tubercles on the anterior and posterior edges with more tubercles on the posterior than on the anterior edges, and show similar ornamentation of the enamel of the crown. There are up to eight major tubercles on at least some teeth in each species.

The teeth of *L. denticrenatus* differ from those of *K. onamata* and *P. vasconum* by having widely separated roots with a cleft between them extending dorsally about halfway up into the crown, thus giving the crown a markedly U-shaped appearance. They also differ by having roots apparently more bulbous and inflated with a distinct groove below the enamel crown incised into the root at the level of the gum line on the lingual side, and in the posterior teeth being situated on the external surface of the ramus and tilted outward.

Phococetus vasconum and *K. onamata* were classified as dorudontids by Kellogg (1936) and most subsequent workers have followed this association. The teeth of *K. onamata* are morphologically derivable from those of primitive dorudontines, and its late time of occurrence is appropriate for possible lineal descent from some archaeocete with three-rooted teeth. The crowns of the teeth of *L. denticrenatus* are morphologically derivable from those of *K. onamata*, but the roots are widely separated like those of dorudontines thus *K. onamata* is a later, highly derived dorudontine, probably related to but not directly descended from an earlier dorudontine lineage ancestral to *L. denticrenatus*. Given the paucity of the fossil record for this time, there may well have been a much greater diversity of Archaeoceti (e.g. Kumar and Sahni 1986) into a variety of niches and habitats than has heretofore been suspected. One such lineage may ultimately be found to be in the direct ancestry of *L. denticrenatus*.

There are at least eight major and minor tubercles in at least some cheekteeth in the new species *L. denticrenatus*. This number, and the large size and prominence of the tubercles, would not preclude the species having a close relationship (sister-group status) with the lineage that gave rise to living mysticetes, following Kuekenthal's (1893) hypothesis about the number and nature of tooth buds posterior to the anterior four anlagen in mysticete embryos. There have been many descriptions of vestigial teeth in the gums of fetal mysticetes (Eschricht 1849; Julin 1880; Pouchet and Chabry 1882, 1884; Weber 1886; Turner 1912, p. 23; Ridewood 1922; Dissel-Scherft and Vervoort 1954) but Kuekenthal (1891) was the first cetologist to attempt well-founded generalizations of their significance. Kuekenthal (1893) studied many embryos and concluded that in early growth, initially fewer teeth with a large number of cusps divide in the course of ontogenetic development into numerous teeth, each corresponding to a cusp. He found the total, including the anterior four buds that had not divided, to be 53 in each half jaw. Abel (1913) related this number to the typical number of seven serrations of archaeocete teeth times the number of cheekteeth, or 49, plus the four anterior teeth for a matching total of 53. This may be too simple a story, however. Arvy (1977) cited these references to anomalies in mysticete teeth, but did not reference the important work of Karlsen (1962), who found tooth germs present in the upper and lower jaws of mysticetes and observed fusion, not fission, in the tooth germs.

Comparison of the teeth of *L. denticrenatus* with those of dorudontines such as *Zygorhiza kochii* (e.g. Kellogg 1936, pls 12–13) indicate that the teeth of the former could be morphologically derived from the latter. The crown is relatively low with eight denticles separated as much as or greater than in *K. onamata* and *P. vasconum*, the roots are widely separated, and they taper distally from their greatest diameter proximate to the crown. Fordyce (1989) described an incomplete dentary of a problematic "?Mysticeti" with large, more deeply inserted teeth than those in *Z. kochii*, with embrasure pits placed more lingually, and an externally inflated horizontal ramus with a very large mandibular canal. The large mandibular canal, extreme external inflation of the horizontal mandibular ramus, close spacing of the cheekteeth and the nature of the occlusion with upper teeth inferred from the embrasure pits all indicate that the species represented by this fossil differs substantially from *L. denticrenatus*. There are some similarities in the placement of the teeth external to the dorsal crest of the ramus, and in the size, distinctness and separation of the denticles on the crowns. However the denticles appear to curve so their tips are directed more dorsally, as in dorudontines and *K. onomata*, than in *L. denticrenatus*. The root of the first premolar (Fordyce 1989, figure 10) appears to be largest in diameter at approximately midlength as in cheekteeth of *K. onomata*.

It is clear from my examination of holotypes, the original and subsequent descriptions of the holotypes and the interpretations of other workers that *Kekenodon onomata* and *Mammalodon colliveri* are valid species and genera of toothed cetaceans of uncertain affinities. I believe that they each represent clades derived from Archaeoceti but not closely related to Odontoceti and only distantly related to the Llanocetidae. Thus I here erect new family-groups to receive them;

Class Mammalia Linnaeus 1758

Order Cetacea Brisson 1762

Suborder Archaeoceti Flower 1883

Superfamily Basilosauroidea (Cope 1868) new rank

Family *incertae sedis*

Subfamily **Kekenodontinae**, new subfamily

Type genus:
Kekenodon Hector 1881, which has as its type species *Kekenodon onomata* Hector 1881, holotype no. NMNZ Ma. 306 (old number Ma.69, cited and specimens enumerated by Kellogg 1936, p.227).

Diagnosis:
Large basilosaurid cetacean with dorudontine affinities having large cheekteeth with up to eight major tubercles or denticles situated anteroposteriorly around entire half circumference of prominent laterally compressed crown, tubercles closely spaced and directed mainly dorsally, enamel ornamented with vertical striae, cingulum weak or absent, most cheekteeth double-rooted, some posterior cheekteeth three-rooted, roots of cheekteeth united for much of length and closely approximated at extremities, inflation or greatest diameter at approximately midlength of root; periotic with deep external notch at base of the anterior process, broad furrow on anterior half of ventral surface of pars cochlearis, groove for tensor tympani muscle narrow and reduced, and fossa for reception of head of malleus broad with abruptly elevated anterior and external edges; tympanic bulla similar to that of *Dorudon intermedius* (Dart 1923).

Included genera:
Kekenodon Hector 1881, *Phococetus* Gervais 1876.

Suborder Mysticeti Flower 1864

Infraorder *incertae sedis*

Family **Mammalodontidae**, new family

Type genus:
Mammalodon Pritchard 1939, which has as its type species *Mammalodon colliveri* Pritchard 1939, holotype no. MUGD 1874. A separate tooth, NMV P17535, part of the original describer's hypodigm, is treated as a syntype by Mahoney and Ride (1975, p.160).

Diagnosis:
Small cetacean with long, low, and little-telescoped skull, broad and flat palate having an upper tooth row slightly convex externally, no well-developed palatal grooves, bones of rostrum sutured very loosely, mandible without a bony symphysis, dentition heterodont, not polydont, dentary with alveoli for 11 teeth, large cheekteeth closely appressed in dentary, lower cheekteeth double-rooted with roots united over most of length, crowns with multiple cusps, ornamentation finely fluted or wrinkled, positive occlusion of upper and lower teeth as judged from marked horizontal wear of crowns of lower cheekteeth along one plane , dentary teeth reclined posteriorly in marked rake of approximately 20°, dentary with large coronoid process rising above level of tooth crowns, mandibular canal large in diameter, extending posteriorly to comprise excavation on internal surface of mandibular condyle, dorsoventral height of mandibular canal approximating height of coronoid process above canal at level of coronoid process, mandibular condyle projecting posteriorly from longitudinal axis of mandibular ramus.

Included genera:
Mammalodon Pritchard 1939.

The current status of the classification of the Cetacea based on existing literature was given by Barnes et al. (1985) The Archaeoceti needs revision. With the description of the Remingtonocetidae, it is clear that a wide diversity of species greatly different in morphology is being referred to three families of mammals. I here raise all three families to superfamily rank: **Protocetoidea**, **Remingtonocetoidea**, and **Basilosauroidea**. My earlier view of the Mysticeti as a clade of mammals defined by the presence of baleen and the loss of teeth clearly does not accord with evidence from the fossil record of whales with the cranial architecture of extant baleen whales but with functional teeth in the adult. Accordingly, I here name the **Chaeomysticeti** or true baleen whales [Gr. *chaios*, genuine, true, good] encompassing living Mysticeti and defined as cetaceans with baleen and without a functional dentiton in the adult stage. Within the Chaeomysticeti I retain the Superfamilies Balaenoidea and Balaenopteroidea first proposed by J. E. Gray in the *Synopsis* of 1868, characterized by the useful diagnoses of Rice (1984). The relationships of *Eschrichtius robustus* have been well studied since Gray separated it from other living species at the family level, and Weber (1904) gave it monotypic family status. Most subsequent work (Barnes and McLeod 1984) continues to recognize its intermediate position between the right and finner whales. My view is that the species represents a lineage long separated from the right and finner whales, and some highly derived features related to feeding specializations tend to indicate closer relationship to other living species than is warranted. I here recognize the family at the new rank of Superfamily **Eschrichtioidea**, with a diagnosis as given by Rice (1984). My view of the relationships between previously described family–group taxa and higher categories and the new taxa described above is shown in the classification below, with standard citations to original authorship and emended rank or context:

- Order Cetacea Brisson 1762
  - Suborder Archaeoceti Flower 1883
    - Superfamily Protocetoidea (Stromer 1908) new rank
      - Family Protocetidae Stromer 1908
    - Superfamily Remingtonocetoidea (Kumar and Sahni 1986) new rank
      - Family Remingtonocetidae Kumar and Sahni 1986
    - Superfamily Basilosauroidea (Cope 1868) new rank
      - Family Basilosauridae Cope 1868
        - Subfamily Dorudontinae (Miller 1923) Slijper 1936
        - Subfamily Basilosaurinae (Cope 1868) Barnes and Mitchell 1978
      - Family *incertae sedis*
        - Subfamily Kekenodontinae new subfamily
  - Suborder Mysticeti Flower 1864 (= Mystacoceti)
    - Infraorder Crenataceti new infraorder
      - Family Llanocetidae new family
    - Infraorder *incertae sedis*
      - Family Aetiocetidae Emlong 1966
      - Family Mammalodontidae new family
    - Infraorder Chaeomysticeti new infraorder
      - Superfamily Balaenopteroidea Gray 1868
        - Family Cetotheriidae (Brandt 1872) Miller 1923
        - Family Balaenopteridae Gray 1866
          - Subfamily Megapterinae Gray 1866
          - Subfamily Balaenopterinae (Gray 1864) Brandt 1872
      - Superfamily Eschrichtioidea (Ellerman and Morrison-Scott 1951) new rank
        - Family Eschrichtiidae Ellerman and Morrison-Scott 1951 (= Rhachianectidae Weber 1940)
      - Superfamily Balaenoidea Gray 1868
        - Family Balaenidae Gray 1825
        - Family Neobalaenidae (Gray 1874) Miller 1923

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References

ABEL, O. 1913. Die vorfahren der bartenwale. Akad. Wiss., Wien, Denksch. Math.-Naturwiss. Kl. 90: [i–ii] + 1–70 + pls. 1–12.

1919. Die Stämme der Wirbeltiere. Walter de Gruyter and Co., Berlin und Leipzig. [i]–xviii + 1–914.

AGARKOV, G. B., AND B. G. KHOMENKO. 1984. Sravnitelnaia morfologiia cherepnomozgovyikh nervov problema i proishozhdeniia kitoobraznykh. [The comparative morphology of craniocerebral nerves and the problem of origin of cetaceans.] Zool. Zh. 63 (5): 760–765.

ANDREWS, R. C. 1914. Monograph of the Pacific Cetacea. I. The California grey whale *Rhachianectes glacialis* Cope). Mem. Am. Mus. Nat. Hist., New Ser. 5 (Pt.5): 229–287 + pls. 19–27.

ANON. 1987. Major fossil find on Seymour Island. Antarctic 11(5): 210–213.

ARNASON, U. 1974a. Comparative chromosome studies in Cetacea. Hereditas 77: 1–36.

1974b. Phylogeny and speciation in Pinnipedia and Cetacea — a cytogenetic study. Thesis, Institute of Genetics, University of Lund, Sweden. p. [1]–8

1982. Karyotype stability in marine mammals. Cytogenet. Cell Genet. 33: 274–276.

ARVY, L. 1977. Contribution to the knowledge of morphological anomalies in cetacean teeth. Invest. Cetacea, 8: 245–254.

BARNES, L. G. 1985. [Review of:] General features of the paleobiological evolution of Cetacea [by] Guram Andreevich Mchedlidze. Mar. Mam. Sci. 1(1): 90–93.

1987. *Aetiocetus* and *Chonecetus*, primitive Oligocene toothed Mysticetes and the origin of baleen whales. J. Vertebr. Paleontol. 7 (Suppl. No. 3): 10A. [Abstract].

1989. Aetiocetus and Chonecetus (Mammalia: Cetacea); Primitive Oligocene toothed mysticetes and the origin of baleen whales. Fifth Int. Theriological Congr., Rome, 22–29 August 1989, Abst. Pap. Posters, Vol. 1, p. 479 [Abstract].

BARNES, L. G. AND S. A. MCLEOD. 1984. The fossil record and phyletic relationships of gray whales, p. 3–32. *In* M. L. Jones, S. L. Swartz, and S. Leatherwood, [ed.] The gray whale *Eschrichtius robustus*. Acad. Press, Inc., Orlando.

BARNES, L. G., D. P. DOMNING, AND C. E. RAY. 1985. Status of studies on fossil marine mammals. Mar. Mamm. Sci. 1(1): 15–53.

BARNES, L. G., AND E. MITCHELL. 1978. Cetacea, p. 582–602. *In* V. J. Maglio and H. B. S. Cooke [ed.] Evolution of African mammals. Harvard University Press, Cambridge.

BARRETT-HAMILTON, G. E. H. 1901. Seals. Expédition Antarctique Belge. Résultats du Voyage Du S.Y. Belgica en 1897–1898–1899 sous le commandement de A. De Gerlache De Gomery. Rapports Scientifiques publiés aux frais du Gouvernement Belge, sous la direction de la Commission dc la Belgica. Zoologie. Anvers, Imprimerie J.-E. Buschmann, p. 1–19 + 1 pl.

BEAUREGARD, H. 1883. Recherches sur l'encéphale des balaenides. J.l'Anat. Physiol. 19: 481–516 + pls. 26–31.

BEDDARD, F. E. 1901. Contribution towards a knowledge of the osteology of the pigmy whale (*Neobalaena marginata*). Trans. Zool. Soc. Lond. 16 (Pt. 2): 87–110 + pls. 7–9.

BENEDEN, P. J., VAN, AND P. GERVAIS. 1880. Ostéographie des cétacés vivants et fossiles comprenant la description et l'iconographie du squelette et du système dentaire de ces animaux ainsi que des documents relatifs à leur histoire naturelle. A. Bertrand, Paris. [i–iv] + i–viii + 1–634 p. + 64 pls. (atlas).

BENHAM, W. B. 1937a. Fossil Cetacea of New Zealand. II. — On *Lophocephalus*, a new genus of Zeuglodont Cetacea. Trans. R. Soc. N.Z. 67: 1–7 + pls. 1–3.

1937b. Fossil Cetacea of New Zealand. IV. — Notes on some of the bones of *Kekenodon onamata* Hector. Trans. R. Soc. N.Z. 67: 15–20 + pls. 8–9.

1939. *Mauicetus*: a fossil whale. Nature (Lond.) 143 (3627): 765.

1942. Fossil Cetacea of New Zealand. V. — *Mauicetus*, a generic name substituted for *Lophocephalus* Benham. Trans. R. Soc. N.Z. 71: 260–270 + pls. 44–47.

Bertram, G. C. L. 1940. The biology of the Weddell and crabeater seals with a study of the comparative behaviour of the Pinnipedia. British Museum (Natural History), British Graham Land Expedition 1934–37 Scientific Reports 1 (No. 1): 1–139 + pls. 1–10.

Breathnach, A. S. 1955a. Observations on endocranial casts of recent and fossil cetaceans. J. Anat. 89 (Pt. 4): 532–546 + pl. 1.

1955b. The surface features of the brain of the humpback whale (*Megaptera novaeangliae*). J. Anat. 89 (Pt. 3): 343–354 + pls. 1–2.

Breathnach, A. S. 1960. The cetacean central nervous system. Biol. Rev. 35(2): 187–230.

Burlet, H. M., de. 1914. Zur entwicklungsgeschichte des walschädels. III. Das primordialcranium eines embryo von *Balaenoptera rostrata* (105 mm). Morphol. Jahrb. 49(1): 119–178 + pls. 5–7.

Cederlund, B. A. 1940. A sub-fossil gray whale discovered in Sweden in 1859. An osteological comparison between the Gräsö whale and the recent gray whale, with a testing of Lilljeborg's nominations and a discussion of the priority of name. Zool. Bidr., Uppsala 18: 269-[286] + pls. 1–5.

Dal Piaz, G. 1905. Sugli Avanzi di *Cyrtodelphis sulcatus* dell'arenaria di Belluno. Palaeontographia Italica. Mem. di Paleont. 11: 253–279.

Dart, R. A. 1923. The brain of the Zeuglodontidae (Cetacea). Proc. Zool. Soc. Lond. 1923: 615–654, [p. 648–652 by C. W. Andrews].

Delfortrie, E. 1873. Un Zeuglodon dans les faluns du Sud-Ouest de la France. Soc. Linnéenne Bordeaux. Actes 29: 113–117.

Dissel-Scherft, M. C. van, and W. Vervoort. 1954. Development of the teeth in fetal *Balaenoptera physalus* (L.) (Cetacea, Mystacoceti) I–II. Proc. Kon. Akad. Wet. Amsterdam, Ser. C, 57: 196–210 + 2 pls.

Edinger, T. 1939. A newly discovered "brain" of an old PROTOSIREN. Fauad I University, Cairo. Faculty Sci. Bull. 19: 43–57 + pls. 1–3.

1955. Hearing and smell in Cetacean history. Monatsschr. Psychiatr. Neurol. 129 (1–3): 37–58.

Elliot, D. H. 1988. Tectonic setting and evolution of the James Ross Basin, northern Antarctic Peninsula. Geol. Soc. Am., Mem. 169: 541–555.

Elliot, D. H., and T. A. Trautman. 1982. Lower tertiary strata on Seymour Island, Antarctic Peninsula, p. 287–297. *In* C. Craddock [ed.] Antarctic Geoscience. Symposium on Antarctic Geology and Geophysics, Madison, Wisconsin, U.S.A., August 22–27, 1977. International Union of Geological Sciences, Ser. B, No. 4. University of Wisconsin Press, Madison, Wisconsin.

Elliot, D. H., C. Rinaldi, W. J. Zinsmeister, T. A. Trautman, W. A. Bryant, and R. Del Valle. 1975. Geological investigations on Seymour Island, Antarctic Peninsula. Antarct. J. U.S. 10 (4): 182–186.

Emlong, D. 1966. A new archaic cetacean from the Oligocene of northwest Oregon. University of Oregon, Mus. Nat. Hist., Bull. 3: [i–iv] + 1–51.

Eschricht, D. F. 1849. Zoologisch-anatomisch-physiologische. Untersuchungen über die nordischen Wallthiere. Leopold Voss, Leipzig. [i]–xvi + 1–[206] + pls. 1–14.

Feldmann, R. M., and M. O. Woodburne [ed.] 1988. Geology and Paleontology of Seymour Island, Antarctic Peninsula. Geol. Soc. Am., Mem. 169: [i]–x + 1–566 + 2 fold. maps.

Flynn, T. T. 1948. Description of *Prosqualodon davidi* Flynn, a fossil cetacean from Tasmania. With a note on the microscopic tooth structure by J. T. Carter. Trans. Zool. Soc. Lond. 26, Pt. 2 (1): 153–197 + pls. 1–6.

Fordyce, R. E. 1977a. The development of the Circum-Antarctic current and the evolution of the Mysticeti (Mammalia: Cetacea). Palaeogeogr. Palaeoclimatol. Palaeoecol. 21 (4): 265–271.

1977b. Paleobiology of New Zealand fossil whales and dolphins. Geol. Soc. N.Z. Newsl. 43: 21–23.

1982a. A review of Australian fossil Cetacea. Mem. Natl. Mus. Victoria 43 (1): 43–58.

1982b. Dental anomaly in a fossil Squalodont dolphin from New Zealand, and the evolution of polydonty in whales. N.Z. J. Zool. 9 (4): 419–426.

1984. The early evolution of baleen whales. N.Z. Mar. Sci. Soc. Newsl. 27: 25 [Abstract].

1988. Beginnings of the baleen whale — *Mammalodon colliveri*, p. 54–56. *In* S. Hand and M. Archer [ed.] The Antipodean Ark. Angus and Robertson, North Ryde.

1989. Problematic Early Oligecene toothed whale (Cetacea. ?Mysticeti) from Waikari, North Canterbury, New Zealand. New Zealand. J. Geol. Geophys. 32: 395–400.

Fraser, F. C. 1952. Handbook of R. H. Burne's Cetacean dissections. British Museum (Natural History) London. p. 1–70.

Fraser, F. C., and P. E. Purves. 1960. Hearing in cetaceans. Evolution of the accessory air sacs and the structure and function of the outer and middle ear in recent cetaceans. Bull. Brit. Mus. (Natural History) Zool. 7 (1): [1]–140 + frontis + pls. 1–53.

Friant, M. 1940. Le télencéphale des hippopotamidés. Arch. Mus. Natl. Hist. Nat. (Paris) (Ser. 6)16: 35–54 + pls. 3–4.

1958. Un stade de l'évolution cérébrale du rorqual (*Balaenoptera musculus* L.). Hvalrådets Skr. 42: [1]–[15].

Gervais, P. 1871. Remarques sur l'anatomie des cétacés de la division des balénidés tirées de l'examen des pièces relatives a ces animaux qui sont conservées au muséum. Nouv. Arch. Mus. Hist. Nat. (Paris) 7: 65–146 + pls. 3–10.

Gingerich, P. D., N. A. Wells, D. E. Russell, and S. M. I. Shah. 1983. Origin of whales in epicontinental remnant seas: new evidence from the Early Eocene of Pakistan. Science (Wash., DC) 220 (4595): 403–406.

Gray, J. E. 1868. Synopsis of the species of whales and dolphins in the collection of the British Museum. London: Bernard Quaritch, p. [1]–10 + pls 1–37.

1874. Hand-list of seals, morses, sea-lions, and sea-bears in the British Museum. Taylor and Francis, London. 43 p. + pls. 1–30.

Guldberg, G. A. 1885. Ueber das centralnervensystem der bartenwale. Forhandl. Videnskaps Selsk. Christiania, No. 4, p. 1–154 + pls. 1–5.

Haiduk, M. W., R. J. Baker, L. W. Robbins, and D. A. Schlitter. 1981. Chromosomal evolution in African megachiroptera: G- and C-band assessment of the magnitude of change in similar standard karyotypes. Cytogenet. Cell Genet. 29 (4): 221–232.

Halstead, L. B., and J. A. Middleton. 1976. Fossil vertebrates of Nigeria Part II. Nigerian Field 41 (3): 129–133.

Hector, J. 1881. Notes on New Zealand Cetacea, recent and fossil. Trans. Proc. N.Z. Inst. 1880. 13: 434–436 + pl. 18.

Honigmann, H. L. 1917. Bau und entwicklung des knorpelschädels vom buckelwal. Zoologica (Stuttgart) 27, Pt. 2 (69): [i–x] + 1–85 + pls. 1–2.

Jansen, J. 1953. Studies on the cetacean brain. The gross anatomy of the rhombencephalon of the fin whale [*Balaenoptera physalus* (L.)]. Hvalrådets Skr. 37: [1]–35.

Jansen, J., and J. K. S. Jansen. 1969. The nervous system of cetacea, p. 175–252. *In* H. T. Andersen [ed.] The biology of marine mammals. Academic Press, New York and London.

Jelgersma, G. 1934. Das gehirn der Wassersäugetiere eine anatomische untersuchung. Johann Ambrosius Barth, Leipzig. [i–viii] + 1–238 p.

Julin, C. 1880. Recherches sur l'ossification du maxillaire inférieur et sur la constitution du système dentaire chex le foetus de la *Balaenoptera rostrata*. Archiv. Biol. 1: 75–136 + pls. 2–3.

Karlsen, K. 1962. Development of tooth germs and adjacent structures in the whalebone whale (*Balaenoptera physalus* (L.)) with a contribution to the theories of the mammalian dentition. Hvalrådets Skr. 45: 1–56 + pls. 1–8.

Kellogg, R. 1928. The history of whales — their adaptation to life in the water. Q. Rev. Biol. 3 (1): 29–76 and 3 (2): 174–208.

1936. A review of the Archaeoceti. Carnegie Institution of Washington, Publ. 482: i–xvi + 1–366 + pls. 1–37.

1968. Fossil marine mammals from the Miocene Calvert Formation of Maryland and Virginia. Bull. U.S. Nat. Mus. 247 (Pts. 5–8): [i–iv] + 103–201 + pls. 46–67.

King, J. E. 1961. The feeding mechanism and jaws of the crabeater seal (*Lobodon carcinophagus*). Mammalia 25 (4): 463–466.

Kleinenberg, S. E. 1958. K voprosu o proishozhdenii kitoobraznykh. Doklady Akad. Nauk SSSR 122(5): 950–952.

1959. On the origin of Cetacea. Proc. XV Int. Cong. Zool: 445–447.

Kooyman, G. L. 1981. Crabeater seal *Lobodon carcinophagus* (Hombron and Jacquinot, 1842). Chpt. 9, p. 221–235. *In* S. H. Ridgway and R. J. Harrison [ed.] Handbook of marine mammals. Vol. 2 Seals. Academic Press Inc. (London) Ltd.

Kükenthal, W. 1891. Einige Bemerkungen über die Säugetierbezahnung. Anat. Anz. 6 (13): 364–370.

1893. Vergleichend-Anatomische und Entwickelungsgeschichtliche Untersuchungen an Walthieren. Zweiter Theil. Denkschr. Medicin.-Naturwiss. Ges. Jena. Dritter Band: i–viii + 221–448 p. + pls. 14–25.

Kumar, K., and A. Sahni. 1986. *Remingtonocetus harudiensis*, new combination, a Middle Eocene Archaeocete (Mammalia, Cetacea) from Western Kutch, India. J. Vertebr. Paleontol. 6(4): 326–349.

Lambertsen, R. H. 1983. Internal mechanism of rorqual feeding. J. Mamm. 64 (1): 76–88.

Langworthy, O. R. 1935. The brain of the whalebone whale, *Balaenoptera physalus*. Bull. Johns Hopkins Hospital 57: 143–147.

1938. A study of the surface structure of the brain of the whale (*Balaenoptera physalus* and *Physeter catodon*). J. Comp. Neurol. 28 (1): 1–47.

Laws, R. M. 1977. Seals and whales of the Southern Ocean. R. Soc. Lond., Phil. Trans., Ser. B 279 (963): 81–96.

1983. Antarctica: a convergence of life. New Sci. 99 (1373): 608–616.

Lillie, D. G. 1915. Cetacea. BM(NH), Brit. Antarctic ("Terra Nova") Exped., 1910, Zool. 1(3): 85–124 + pls. 1–8.

Mahoney, J. A., and W. D. L. Ride. 1975. Index to the genera and species of fossil mammalia described from Australia and New Guinea between 1838 and 1968 including citations of type species and primary type specimens. W. Australian Mus. Spec. Publ. No. 6: 1–250.

Marples, B. J. 1949. Two endocranial casts of cetaceans from the Oligocene of New Zealand. Am. J. Sci. 247 (7): 462–471.

1956. Cetotheres (Cetacea) from the Oligocene of New Zealand. Proc. Zool. Soc. Lond. 126: 565–580 + 1 pl.

Mchedlidze, G. A. 1970. Nekotorye obshchie cherty istorii kitoobraznykh. [Some features of the historical development of the Cetacea.] Pt. 1. Tbilisi, Acad. Nauk GSSR, Inst. Paleobiol., Metsniereba Publishers, [1]–[114] + pls. 1–6 + [i].

1977. A review of the historical development of the Cetacea. J. Palaeontol. Soc. India 20: 81–88 + pls. 1–9.

1976. Osnovnye cherty paleobiologicheskoi istorii kitoobraznykh. [General features of the historical development of Cetacea.] Acad. Nauk GSSR, Inst. Paleobiol., Metsniereba Publishers, Tbilisi. [1]–[136] + pls. 1–32 + [i].

1984. General features of the paleobiological evolution of Cetacea. [Osnovnye cherty paleobiologicheskoi istorii kitoobraznykh.] Published for the Smithsonian Institution Libraries and the National Science Foundation, Washington, D.C., by Amerind Publishing Co. Pvt. Ltd., New Delhi. [i]–viii + 1–139 p. + pls. 1–32.

McKay, A. 1882a. Geology of the Waitaki valley and parts of Vincent and Lake countries. Rep. Geol. Explor. 1881, Colonial Mus. and Geol. Surv. Dept., Wellington, N.Z., p. 56–92 + 1 pl. (map).

1882b. On the younger deposits of the Wharekauri basin and the lower Waitaki valley. Rep. Geol. Explor. 1881, Colonial Mus. and Geol. Surv. Dept. Wellington, N.Z., p. 98–106.

McLeod, S. A., R. L. Brownell, Jr., and L. G. Barnes. 1989. The classification of cetaceans: are all whales related? Fifth Int. Theriological Congr., Rome, 22–29 August 1989, Abst. Pap. Posters, Vol. 1, p. 490 [Abstract].

Miller, G. S. 1923. The telescoping of the Cetacean skull. Smithsonian Misc. Collect. 76 (5): 1–[71].

Mitchell, E. 1975. Trophic relationships and competition for food in Northwest Atlantic whales, p. 123–133. *In* M. D. B. Burt [ed.] Proceedings of the Canadian Society of Zoologists Annual Meeting, June 2–5, 1974. 133 p.

Mitchell, E., and A. R. Baker. 1980. Age of reputedly old killer whale, *Orcinus orca*, 'Old Tom' from Eden, Twofold Bay, Australia. Rep. Int. Whaling Comm., Spec. Iss. 3: 143–154.

Morgane, P. J., and M. S. Jacobs. 1972. Comparative anatomy of the Cetacean nervous system, p. 117–244. *In* R. J. Harrison [ed.] Functional anatomy of marine mammals. Vol. 1. Academic Press, London, and New York.

Nemoto, T. 1959. Food of baleen whales with reference to whale movements. Sci. Rep. Whales Res. Inst. 14: 149–290 + pl. 1.

Oelschlaeger, H. A. 1987. *Pakicetus inachus* and the origin of whales and dolphins (Mammalia: Cetacea). Gegenbaurs Morphol. Jahrb., Leipzig 133 (5): 673–685.

Ommanney, F. D. 1932. The vascular networks (Retia Mirabilia) of the fin whale (*Balaenoptera physalus*). Discovery Rep. 5: [327]–362.

Pilleri, G. 1963. Zur vergleichenden morphologie und rangordnung des gehirns von *Delphinapterus* (Beluga) *leucas* Pallas (*Cetacea, Delphinapteridae*). Rev. Suisse Zool. 70 (28): 569–586 + pls. 1–7.

1972. The cerebral anatomy of the Platanistidae (*Platanista gangetica, Platanista indi, Pontoporia blainvillei, Inia geoffrensis*). Invest. Cetacea 4: 44–70 + pls. 1–18.

Pivorunas, A. 1976. A mathematical consideration on the function of baleen plates and their fringes. Sci. Rep. Whales Res. Inst., 28: 37–55.

1977. The fibrocartilage skeleton and related structures of the ventral pouch of Balaenopterid whales. J. Morph. 151 (2): 299–306 + pls. 1–3.

1979. The feeding mechanisms of baleen whales. Amer. Sci. 67 (4): 432–440.

Pouchet, G., and L. Chabry. 1882. Sur l'évolution des dents des Balaenides. Compt. Rend. Acad. Sci. Paris 94 (8): 540–542.

1884. Contribution à l'odontologie des mammifères. J. l'Anat. Physiol. 20: 149–192. [Not seen].

Pritchard, B. G. 1939. On the discovery of a fossil whale in the older tertiaries of Torquay, Victoria. Victorian Nat. 55 (9): 151–159.

Racovitza, E. G. 1900. La vie des animaux et des plantes dans l'Antarctique. Soc. R. Belge de Géog. Bull. 24: 177–230 + 3 maps.

Reinhardt, J. 1869. Ni tavler til oplysning af hvaldyrenes bygning, udførte til utrykte foredrag af afdøde etatsraad Dr. D. F. Eschricht. Vidensk. Selsk. Skr., Ser. 5, naturvidensk. math. Afd., 9, Vol. 1: 1–14 + pls. 1–9.

Rice, D. W. 1984. Cetaceans, p. 447–490. *In* S. Anderson and J. K. Jones, Jr., [eds.] Orders and families of recent mammals of the worlds. John Wiley and Sons, New York, NY.

Ridewood, W. G. 1922. Observations on the skull in foetal specimens of whales of the genera *Megaptera* and *Balaenoptera*. R. Soc. Lond. Phil. Trans. Ser. B, 211: 209–272.

Rothausen, K. 1968. Die systematische Stellung der europäischen Squalodontidae (Odontoceti, Mamm.). Paläont. Z. 42 (1/2): 83–104 + pls. 11–12.

1971. *Cetotheriopsis tobieni* n. sp., der erste paläogene bartenwal (Cetotheriidae, Mysticeti, Mamm.) nördlich des Tethysraumes. Abh. hess. L.-Amt Bodenforsch. 60, Heinz-Tobien-Festschrift, p. 131–148 + pls. 1–3.

Russell, L. S. 1968. A new cetacean from the Oligocene Sooke Formation of Vancouver Island, British Columbia. Can. J. Earth Sci. 5: 929–933 + pls. 1–2.

Sahni, A., and V. P. Mishra. 1972. A new species of *Protocetus* (Cetacea) from the Middle Eocene of Kutch, Western India. Palaeontology 15, Pt. 3: 490–495 + pl. 97.

Scheffer, V. B. 1964. Teeth of the crabeater seal. Bioscience 14 (9): 51 + cover plate.

Schulte, H. von W. 1916. Anatomy of a foetus of *Balaenoptera borealis*. Mem. Am. Mus. Nat. Hist., New Ser. 1 (Pt. 6): 389–502 + pls. 43–57.

Smith, G. E. 1903. The brain of the Archaeoceti. Proc. R. Soc. Lond. 71: 322–331.

Stromer, E. 1908. Die Archaeoceti des ägyptischen Eozäns. Beitr. Paläontol. Geol. Oesterreich-Ungarns Orients 21: 106–178 + pls. 4–7.

True, F. W. 1912. The genera of fossil whalebone whales allied to *Balaenoptera*. Smithsonian Misc. Coll. 59(6): 1–8.

Tullberg, T. 1883. Bau und Entwicklung der Barten bei *Balaenoptera sibbaldii*. Nova Acta Regiae Soc. Sci. Upsaliensis, (ser. 3) Fasc. 2 (3): 1–36 + pls. 1–7.

Turner, W. 1912. The marine mammals in the Anatomical Museum of the University of Edinburgh. Macmillan and Co., London. xv + 207 p. + pls. 1–17.

Van Utrecht, W. L. 1958. Temperaturregulierend Gefäßsysteme in der Haut und anderen epidermalen Strukturen bei Cetaceen. Zool. Anz. 161: 77–82.

1966. On the growth of the baleen plate of the fin whale and the blue whale. Bidr. tot Dierk. 35: 3–38 + 9 pls.

Van Valen, L. 1968. Monophyly or diphyly in the origin of whales. Evolution 22 (1): 37–41.

Walmsley, R. 1938. Some observations on the vascular system of a female fetal finback. Carnegie Institution of Washington, Contrib. Embryol. 27 (164): [107]–178 + pls. 1–5.

Weber, M. 1886. Studien über Säugetiere. Ein beitrag zum frage nach dem ursprung der Cetaceen. Gustav Fischer, Jena. viii + 252 p. + pls. 1–4.

1904. Die Säugetiere. Einführung in die anatomie und systematik der recenten und fossilen Mammalia. Gustav Fischer, Jena, 866 p.

1927–1928. Die Säugetiere. Einführung in die anatomie und systematik der recenten und fossilen Mammalia. Gustav Fischer, Jena. Vol. I. Antomischer Teil, xv + 444 p. [1927, with collaboration of H. M. de Burlet]. Vol. II. Systematischer Teil, xxiv + 898 p. [1928, with collaboration of Othenio Abel].

Whitmore, F. C. jr. and A. E. Sanders. 1976. Review of the Oligocene Cetacea. Syst. Zool. 25 (4): 304–320.

Wiley, E. O. 1981. Phylogenetics: the theory and practice of phylogenetic systematics. John Wiley and Sons, New York, Chichester, Brisbane, Toronto. xv + 439 p.

Wilson, L. E. 1973. A delphinid (Mammalia, Cetacea) from the Miocene of Palos Verdes Hills, California. Univ. Calif. Publ. Geol. Sci. 103: [i–vi] + [1]–34.

Wilson, R. B. 1933. The anatomy of the brain of the whale (*Balaenoptera sulfurea*). J. Comp. Neurol. 58 (2): 419–480.

Wiman, C. 1905. Uber die alttertiaren Vertebraten der Seymourinsel. Schwedischen Sudpolar-Expedition 1901–1903, Vol. 3, Geol. Palaontol.: 1–37 p. + pls. 1–8.

Winge, H. 1921. A review of the interrelationships of the Cetacea. Smithsonian Misc. Collect. 72 (8): 1–97.

Woodburne, M. O., and W. J. Zinsmeister. 1984. The first land mammal from Antarctica and its biogeographic implications. J. Paleont. 58(4): 913–948.

Yablokov, A. V. 1964. Konvergenciya ili parallelizm v razvitii kitoobraznykh. Akad. Nauk SSSR, Paleont. Zhur. No. 1: 97–196.

1965. Convergence or parallelism in the evolution of cetaceans. Int. Geol. Rev. 7(8): 1461–1468.

ZHEMKOVA, Z. P. 1965. O proiskhozhdenii kitoobraznykh (*Cetacea*). Zool. Zh. 44 (10): 1546–1552. [In Russian, English summary].

1971. On the origin of *Cetacea*. Dept. Secretary of State of Canada, Transl. Bur., Fisheries Research Board of Canada, Transl. Ser. No. 1778, p. [i–ii] + 1–20 typescript.

ZINSMEISTER, W. J., AND H. H. CAMACHO. 1982. Late Eocene (to possibly Earliest Oligocene) molluscan fauna of the La Meseta Formation of Seymour Island, Antarctic Peninsula. p. 299–304. *In* C. Craddock [ed.] Antarctic Geoscience, Symposium on Antarctic Geology and Geophysics, Madison, Wisconsin, U.S.A., August 22–27, 1977. International Union of Geological Sciences, Ser. B, No. 4. University of Wisconsin Press, Madison, Wisconsin.

ANNOUNCEMENT/AVIS

International Conference on the Environmental Management of Enclosed Coastal Seas '90

August 3–6, 1990

Kobe, Japan

For a long time, human beings have made good use of the areas in and around coastal sites such as inland seas, atolls, and archipelagos, for fisheries and industrial activities as well as for waterborne traffic and places of recreation, since those areas are endowed with excellent natural environments. Enclosed coastal seas, however, are vulnerable to contamination because of the poor exchange of sea water. The many regions of the worlds endowed with enclosed coastal seas are now confronted with the problems of environmental preservation and appropriate use. Thus we propose convening "The International Conference on the Environmental Management of Enclosed Coastal Seas '90 (EMECS '90)" in Kobe on the Seto Inland Sea, one of the typical enclosed coastal seas located in Japan.

Scope and Objectives:

(1) To promote the exchange of information and technology on environmental management of enclosed coastal seas.
- (a) Present state of environmental pollution
- (b) Measures against water pollution and their effectiveness
- (c) Control on pollution load
- (d) Laws and regulations relating to environmental protection and development coordination
- (e) Assistance to developing countries related to environmental protection
- (f) World network for technological exchange, research, and studies
- (g) Possibility of the establishment of a joint research organization by two or more countries

(2) To explore a course of environmental protection and appropriate use of enclosed coastal seas.
- (a) Measures for comprehensive management of sea waters as well as coastal areas.
- (b) Coastal sea area utilization and ecological system preservation
- (c) Fishery resource preservation and utilization
- (d) Resort development and natural environment
- (e) Development of waterfronts and preservation of natural coasts
- (f) Creation of new environments

(3) To explore a cultural norm for the environmental protection of enclosed coastal seas from the historical as well as other perspectives.
- (a) Relations between human activities and coastal sea environments
- (b) Nongovernmental activities for environmental protection

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International Conference on the Environmental Management of Enclosed Coastal Seas '90

du 3 au 6 août 1990

Kōbe, Japon

De temps immémorial, l'espèce humaine a fait usage des mers côtières fermées et des zones environnantes comme les atolls et les archipels dont il s'est servi autant pour la pêche et des activités industrielles, que pour le trafic maritime et les activités récréatives, étant donné que ces régions bénéficient d'excellents environnements naturels. Toutefois, les mers côtières fermées sont susceptibles à la contamination à cause de la faible circulation d'eau salée. On doit donc se pencher sur les problèmes de protection de l'environnement et d'utilisation appropriée des nombreuses régions du globe dotées de mers côtières fermées. À cette fin, nous convoquons la *International Conference on the Environmental Management of Enclosed Seas '90 (EMECS '90)* qui aura lieu à Kōbe (Japon), ville située sur le littoral de la mer Intérieure ou Seto Naikai qui est un exemple typique d'une mer côtière fermée.

Portée et objectifs :

(1) Promouvoir l'échange d'information et de techniques liées à la gestion de l'environnement des mers côtières fermées, soit
- (a) le niveau actuel de pollution environnementale,
- (b) les mesures prises contre la pollution de l'eau et leur efficacité,
- (c) le contrôle de la charge de polluants,
- (d) les lois et les règlements ayant trait à la protection de l'environnement et à la coordination de l'aménagement,
- (e) l'aide aux pays en voie de développement pour la protection de l'environnement,
- (f) un réseau international d'échange d'information et de technologie et de réalisation d'études et de recherches,
- (g) la création d'un organisme de recherche mixte par deux pays ou plus.

(2) Étudier un programme de protection de l'environnement et d'utilisation appropriée des mers côtières fermées, soit
- (a) des mesures de gestion globale des eaux marines ainsi que des zones côtières,
- (b) l'utilisation des zones marines côtières et la protection de l'écosystème,
- (c) l'utilisation et la protection des ressources halieutiques,
- (d) la mise en valeur de lieux de villégiature et les environnements naturels,
- (e) l'aménagement du secteur riverain et la protection des zones côtières naturelles,
- (f) la création de nouveaux environnements.

(3) Évaluer une norme culturelle pour la protection de l'environnement des mers côtières fermées, au niveau historique et autres perspectives, soit

(c) Educational activities for environmental protection

Note: Conference languages will be in Japanese and English with simultaneous translation.

Further information: Secretariat, Executive Committee of the International Conference on the Environmental Management of Enclosed Coastal Seas 10-1, 5-chome, Shimoyamate-dori, Chuo-ku, Kobe 650, Japan. Phone: (078) 341-7711 ext. 4115, 4113. (078) 351-2039 (after 6 p.m. only). FAX: (078) 371-4354.

(a) les relations entre les activités humaines et les environnements marins côtiers,
(b) les activités privées axées sur la protection de l'environnement,
(c) les efforts d'éducation pour la protection de l'environnement.

Note : Les délibérations auront lieu en japonais et en anglais, avec traduction simultanée.

Pour renseignements, veuillez contacter : Le Secrétariat du Comité exécutif, International Conference on the Environmental Management of Enclosed Coastal Seas 10-1, 5-chome, Shimoyamate-dori, Chuo-ku, Kōbe 650, Japon Téléphone : (078) 341–7711 poste 4115, 4113 (078) 351–2039 (après 18 h), Fax : (078) 371–4354.

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Journal canadien des sciences halieutiques et aquatiques

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